POLYCHLORINATED DIBENZOFURANS:

CRITERIA FOR THEIR EFFECTS ON HUMANS AND THE ENVIRONMENT

BY

M.F. MITCHELL, H.A. MCLEOD AND J.R. ROBERTS

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NRCC ASSOCIATE COMMITTEE ON SCIENTIFIC CRITERIA

FOR

ENVIRONMENTAL QUALITY

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M.F. Mitchell, H.A. McLeod and J.R. Roberts

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All documents published by the Associate Committee are published in both French and English.

TERMS OF REFERENCE OF THE SUBCOMMITTEE ON PESTICIDES AND INDUSTRIAL ORGANIC CHEMICALS

(1) The preparation of reports critically evaluating and collating the available cause-effect relationships, which permit the estimation of the environmental risks associated with the use of synthetic organic chemicals in Canada - these documents will provide criteria for the evaluation of both present and future use patterns, and

(2) the identification of gaps in available criteria and preparation of recommendations for future research in these areas.

To meet this mandate, the Subcommittee has identified a number of priority compounds for consideration. Comprehensive reports are prepared to bring into perspective the diverse and somewhat obscure observations found in the literature. The documents consist of reviews, summarizing the available criteria and the research required. They also contain as supporting material the available data on which the overview is based.

The mandate of the Subcommittee does not include the setting of objectives and standards, the development of risk assessment, or the establishment of registered uses for pesticides in Canada. Therefore, it does not permit consideration of the engineering, economic, or legal aspects. The Subcommittee is only concerned with scientific information and environmental criteria. The documents do provide one of the components necessary in the consideration and development of risk assessments. Other agencies, both federal and provincial, have the actual responsibility for making decisions about the uses of specific synthetic organic chemicals in Canada, and hence they have the task of bringing about an acceptable balance between the risks and benefits associated with the use of such chemicals by our society.

Inquiries concerning the activities of the Subcommittee should be directed to the Environmental Secretariat of the National Research Council of Canada, Ottawa, Canada.

NOTICE ON SUBCOMMITTEE MONOGRAPHS CONTAINING INDUSTRIAL BIOTEST MATERIAL

Picloram: The Effects of Its Use as a Herbicide on Environmental Quality. 1974. NRCC No. 13684

Endosulfan: Its Effects on Environmental Quality. 1975. NRCC No. 14098

Fenitrothion: The Effects of Its Use on Environmental Quality and Its Chemistry. 1975. NRCC No. 14104

Fenitrothion: The Long Term Effects of Its Use in Forest Ecosystems - Current Status. 1977. NRCC No. 15389

Proceedings of a Symposium on Fenitrothion: The Long-Term Effects of Its Use in Forest Ecosystems. 1977. NRCC No. 16073

Bacillus thuringiensis: Its Effects on Environmental Quality. 1976. NRCC No. 15385

Polychlorinated Biphenyls: Biological Criteria for an Assessment of Their Effects on Environmental Quality. 1978. NRCC No. 16077

Ecotoxicology of Chlorpyrifos. 1978. NRCC No. 16079

Carbofuran: Criteria for Interpreting the Effects of Its Use on Environmental Quality. 1979. NRCC No. 16740

Approximately four years ago, it was recognized by Canadian and U.S. authorities that irregularities existed in some reports of animal studies carried out by Industrial Biotest Laboratories Incorporated of Northbrook, Illinois. A cooperative effort of Health and Welfare Canada and the U.S. Environmental Protection Agency to validate the studies indicates that safety evaluations of 97 pesticides are supported wholely or in part by studies from this contract laboratory. Audits of the individual studies indicate that a significant portion are invalid. These agencies have requested new studies when information central to the assessment of human safety is "suspect". Specific details of the concerns that form the basis of these requests are unavailable.

The following monographs of the NRC's Subcommittee for Pesticides and Industrial Organic Chemicals are affected: picloram, endosulfan, fenitrothion, Bacillus thuringiensis, polychlorinated biphenyls, chloropyrifos and carbofuran. These documents contain some toxicological studies conducted by IBT, which must be considered as "suspect" in light of the present situation. Hence, until the matter of the validation has been clarified, particular caution should be maintained to avoid the use of criteria developed on the basis of the IBT material. The Subcommittee is presently revising and, where necessary, updating the affected monographs and the update on the fenitrothion documents (NRCC No. 22492) is now published.

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ABBREVIATIONS

AHH - aryl hydrocarbon hydroxylase

BW - body weight

CI - chemical ionization

CP - chlorinated phenol

d - day

D_2CDF - dichlorinated dibenzofuran

EI - electron impact

LRGC - low-resolution gas chromatography

F - female

GLC - gas-liquid chromatography

HCBz - hexachlorobenzene

H_7CDF - heptachlorinated dibenzofuran

H_6CDF - hexachlorinated dibenzofuran

HRGC - high-resolution gas chromatography

i.p. - intraperitoneal

i.v. - intravenous

LOEL - lowest observed-effect level

M - male

M_1CDF - monochlorinated dibenzofuran

MFO - mixed-function oxidase

MS - mass spectrometry (er)

NCI - negative chemical ionization

ND - not detected

ABBREVIATIONS (CONT'D)

NOEL - no observed-effect level

O_8CDF - octachlorinated dibenzofuran

PBB - polybrominated biphenyl

PCB - polychlorinated biphenyl

PCBz - polychlorinated benzene

PCDD - polychlorinated dibenzo-p-dioxin

PCDF - polychlorinated dibenzofuran

P_5CDF - pentachlorinated dibenzofuran

PCDPE - polychlorinated diphenylether

PCN - polychlorinated naphthalene

P_5CP - pentachlorophenol

PCQ - polychlorinated quaterphenyl

PCT - polychlorinated terphenyl

RP - reverse phase

SCE - sister chromatid exchange

SD - Sprague Dawley

T_3CDF - trichlorinated dibenzofuran

T_4CDF - tetrachlorinated dibenzofuran

wk - week

1.0 CRITIQUE AND RECOMMENDATIONS[α]

The polychlorinated dibenzofurans (PCDFs) are a large family of lipophilic, halogenated aryl hydrocarbons (135 congeners; Figure 1-1). They are widely distributed in the environment, possibly in concentrations higher than the well-publicized polychlorinated dibenzo-p-dioxins (PCDDs).

The few PCDF congeners that have been tested differ in toxic potential. Their acute toxicity pattern parallels that of similarly substituted PCDDs and the acute toxicity of the PCDF congeners tested is generally only about one half to one tenth that of the parallel PCDDs. Hence, like the PCDDs, many of the congeners must be considered extremely toxic.

The extreme toxicity of some of the PCDF congeners necessitates analytical methods with very high sensitivity and specificity. Only with sensitive, isomer-specific analysis can the potential hazard of a particular PCDF mixture present in the environment be estimated.

1.1 LIMITATIONS TO ANALYTICAL TECHNIQUES (discussed in detail in Chapter 3)

Most techniques offer no certainty that, when a PCDF is reported in a matrix, it has been correctly identified and accurately measured. Too often, the bias is to underestimate concentrations. Thus, an understanding of the limitations of analytical results is crucial to their interpretation.

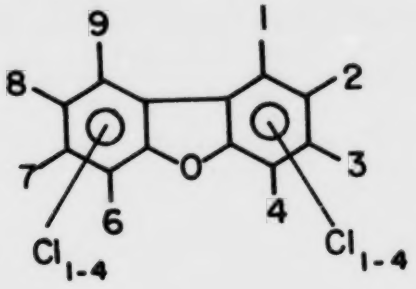
An NRCC expert panel (1981b) presented a highly technical review and assessment of the then current (to December 1980) methodology for the analysis of PCDDs. In general, the comments and recommendations of that panel (Appendix VI) apply to the PCDFs. The problems of separation are even greater for the PCDFs since there are more PCDF than PCDD congeners (135 vs. 75). The removal of possible artifacts, such as interfering compounds, is critical to any technique so that concentrations can be accurately determined. This is particularly difficult at low PCDF concentrations. Sufficient confirmation of structures is also essential.

[α] The intent of this monograph is not to provide an exhaustive review of all existing literature. References retrieved from the CAN/OLE data bases up to April 1984 were considered, along with other data made available to the authors. Some pertinent data, submitted to the authors by May 1984, were added during review.

Figure 1-1. Numbering and nomenclature system for chlorine atoms of the PCDF and PCDD congeners[α].

PCDFs

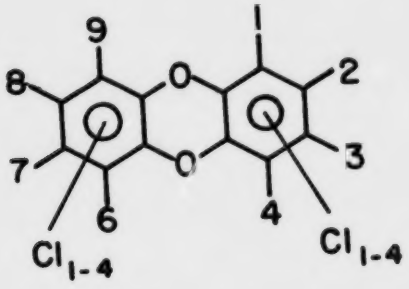
135 congeners[α]



Lateral ring positions are 2,3,7 and 8

PCDDs

75 congeners[α]



Homologue[α]	Abbreviation	Number of[α] Isomers
Monochlorinated dibenzofuran	M_1CDF	4
Dichlorinated dibenzofuran	D_2CDF	16
Trichlorinated dibenzofuran	T_3CDF	28
Tetrachlorinated dibenzofuran	T_4CDF	38
Pentachlorinated dibenzofuran	P_5CDF	28
Hexachlorinated dibenzofuran	H_6CDF	16
Heptachlorinated dibenzofuran	H_7CDF	4
Octachlorinated dibenzofuran	O_8CDF	1
Monochlorinated dibenzo-ρ-dioxin	M_1CDD	2
Dichlorinated dibenzo-ρ-dioxin	D_2CDD	10
Trichlorinated dibenzo-ρ-dioxin	T_3CDD	14
Tetrachlorinated dibenzo-ρ-dioxin	T_4CDD	22
Pentachlorinated dibenzo-ρ-dioxin	P_5CDD	14
Hexachlorinated dibenzo-ρ-dioxin	H_6CDD	10
Heptachlorinated dibenzo-ρ-dioxin	H_7CDD	2
Octachlorinated dibenzo-ρ-dioxin	O_8CDD	1

[α] Congener denotes any isomer of any homologue. Homologue defines a group of isomers with the same number of chlorine atoms. An isomer is defined by the arrangement of chlorine atoms within the homologue.

Major advances in analytical abilities for the PCDFs during the last two years include the development and extensive use of chemical ionization (CI) and the use of CI in a triple-quadrupole mode. Detection limits in the range of $ng.kg^{-1}$ (compared to the more usual $\mu g.kg^{-1}$) have been obtained using these techniques. The techniques have not been demonstrated to be optimized for specificity. A particular problem is the lack of validation.

Authenticated material is needed to evaluate data. Most of the PCDF congeners have now been synthesized, making specific confirmation at least theoretically possible. However, many standards are still difficult and time consuming to produce, making them expensive. Some exist only in extremely limited quantities or as mixtures and are not universally available.

Inter- and intralaboratory quality assurance programs could help establish confidence in data. The potential for contamination is so high that it is a ubiquitous problem and random spot-checks on precision and accuracy are essential. Collaborative studies in different laboratories can increase the confidence of reported data.

Guidelines exist for sampling and extraction techniques. However, the exact nature of a sample and the analytical techniques used are not always specified in reports. Thus it is often unclear whether a sample and determined result is representative of its source.

1.2 SOURCES AND LEVELS OF PCDFs IN THE ENVIRONMENT (discussed in detail in Chapter 2)

While it is generally accepted that PCDDs and PCDFs occur ubiquitously in the industrialized environment, there are few data on concentrations in the Canadian environment. It is difficult to estimate the total amount of PCDFs entering the Canadian environment, and still more difficult to estimate the amounts of particular congeners. Different sources release different patterns of congeners to the environment. However, the partitioning and degradation patterns in the environment, mainly determined by the solubility and persistence of individual congeners, confound any attempt to relate congener patterns to source. It is thus impossible to relate levels of PCDFs in the environment to specific sources.

1.2.1 PCDF LEVELS IN THE ENVIRONMENT

At present, few environmental matrices have been monitored for PCDFs. They have been detected in North American fish and gulls (up to ~100 $\mu g.kg^{-1}$, including significant amounts of the relatively more toxic congeners), other aquatic organisms, aquatic sediments and in other food destined for human consumption (H_6CDF and O_8CDF at up to 0.4 $\mu g.kg^{-1}$ in gelatin, 0.04 to 0.13 $ng.kg^{-1}$ in chicken liver).

The potential impact of PCDFs in the environment cannot be assessed until the levels of specific congener mixtures in specific environmental compartments (matrices), food sources and human tissue are determined so that relatively high exposure situations can be identified. It is also important to know the biological relevance of the contamination, i.e. the relative toxic activity of the congener mix and the bioavailability from the matrix.

Although the confirmation of "detected" PCDFs is important for regulatory purposes, the practical number of congener-specific determinations is limited by the considerable time and money needed. Attention obviously should be focused on high-risk situations.

Rather than expanding the monitoring and assessment of specific PCDF congener concentrations in the environment, with the imposed limitations, it would be more useful to concentrate on assessing the biological activity of the congener mixture in a matrix. This would still involve some analytical precision in purifying samples to a known and consistent level and would require an improved understanding of toxic mechanisms (see Section 1.5).

1.2.2 PCDF SOURCES

As discussed, environmental partitioning patterns make it almost impossible to extrapolate between environmental levels and sources. An exception could be where a congener is not produced by a specific source. For example, the detection of 2,3,7,8-T_4CDF near a wood processing plant would suggest pentachlorophenol (P_5CP) is not the only source.

Obviously, attention should be focused on cleanup of the environment. Priorities still need to be established. It would be useful to rank predicted human intakes of PCDF mixtures, relative to the sources, in terms of the toxicity of the mixtures. This would involve a better understanding of the relation of toxicity to structure (see Section 1.5) as well as documenting typical intake from each source (e.g. industrial effluents, incinerators, food, etc.). Environment Canada has already undertaken a useful first step by initiating the development of an inventory of Canadian sources of PCDDs and PCDFs.

Despite all these limitations, it is possible to gain a general idea of the importance of some particular sources and to estimate the relative magnitude of the problems.

Polychlorinated biphenyls (PCBs) (see Section 2.3.1)

One major documented source of PCDFs in North America is PCBs, albeit PCBs are no longer being manufactured. At least 3 tonnes of PCB-

derived PCDFs may have entered the North American environment since 1930. There were at least 1.7×10^7 kg of PCBs in the 1982 Canadian national inventory. This may include 85 kg of PCDFs, as much as half of which could be the highly toxic 2,3,7,8-isomer. In PCBs that have been subjected to heat the levels of PCDFs can be increased. Under extreme conditions, PCDFs can be formed in very high concentrations. Transformer and condenser applications accounted for more than 70% of the total Canadian PCB sales. Fires in buildings that contain PCB-electrical equipment are of particular concern.

Very high PCDF concentrations have been reported when PCBs are burned at temperatures up to 800°C; the discussion of satisfactory disposal methods for PCBs is outside the mandate of this document.

Chlorinated Phenols and Related Compounds (see Section 2.3.2)

The chlorinated phenols are another major source of PCDFs. As much as 800 kg of total PCDFs derived from P_5CP could enter the Canadian environment each year. H_7CDFs and O_8CDF are the major PCDF contaminants. The highly toxic 2,3,7,8-isomer, if it occurs at all, is a very minor constituent in this source.

As manufacturers are not required to document PCDF levels in formulations, the contribution from phenoxy herbicides is difficult to assess.

Combustion (see Section 2.3.3)

An estimated 88 kg of total PCDF per year could enter the Canadian environment as a result of municipal combustion. In general, total PCDF levels in incinerator emissions are equal to or higher than PCDD levels.

The congener pattern for combustion-derived PCDFs is different from that for combustion-derived PCDDs. The hexa- and hepta-PCDD homologues are prominent while the more toxic PCDD congeners are present only as minor constituents. In the case of the PCDFs, there are relatively higher amounts of the lower chlorinated homologues, including the most toxic isomers as major constituents.

1.3 FATE AND PERSISTENCE (discussed in detail in Chapter 4)

Almost no data are available for evaluating the environmental behavior of PCDFs. Photodegradation in sunlight is apparently quite slow but exhaustive studies do not exist. There are no data on bacterial or fungal catabolism. Because vapor pressures and

solubilities have not been determined, the volatilization potential cannot be predicted.

Because they are lipophilic, it is reasonable that PCDFs will bioaccumulate. This is supported by their detection in fish. Differences in the lipophilicity of the congeners will affect partitioning patterns and hence complicate the interpretation of residue data. Models could be of some use in making gross, initial predictions of the environmental distribution and persistence of homologues. However, the use of models is limited by the lack of data on basic physical and chemical parameters.

1.4 METABOLIC DISPOSITION (discussed in detail in Chapter 6)

Studies comparing guinea pigs and other mammals suggest that the rate at which a species can eliminate 2,3,7,8-T_4CDF is inversely related to its toxic susceptibility to 2,3,7,8-T_4CDF. The rate of elimination of a specific congener, which is primarily dependent on the rate of its metabolism in the liver, is apparently determined by the structure of the congener. In Yusho patients, all congeners with two vicinal hydrogens[α] in at least one ring were excreted, while most of the highly retained congeners had chlorine in 3 or 4 of the lateral positions and no vicinal hydrogens. However, hepatic distribution does not appear to be directly associated with the acute toxicity of specific congeners; for example, although 2,3,7,8-T_4CDF and 2,3,4,7,8-P_5CDF are similar in their structure, MFO inducibility and toxicity in rats, they are retained to very different extents in rat liver.

PCDFs are transferred across the placenta in female mice, and in larger amounts to the offspring via milk. Certain congeners are preferentially excreted by the dams, or retained in the liver or transferred to the offspring.

Metabolism of the PCDFs does occur. In contrast to the PCDDs, where hydroxyderivatives substituted only at the 2,3 positions have been isolated, PCDF metabolites apparently show a wider substitution pattern.

1.5 TOXICOLOGY (discussed in detail in Chapter 7)

Only limited toxicological data are available for 2,3,7,8-T_4CDF. Few data exist for any of the other PCDF congeners. This makes assessment difficult. On the basis of the PCDDs, a range through 10^5

[α] Vicinal hydrogens are substituted in adjacent sites in a molecule.

could be expected for the lethality of the congeners. Obviously, it is not practical to assess the toxic effects of all 135 congeners in laboratory animals. It is thus important to enhance our understanding of the mechanism of action. Ideally, this understanding should advance to a stage where the relative toxicity of a congener can be predicted from its structure.

The most potent PCDD congeners, in terms of toxicity, have two common structural features:

a) halogen atoms occupy at least 3 of the lateral ring positions (2,3,7 and 8; Figure 1-1); and

b) at least one ring position is non-halogenated.

The absence of vicinal hydrogens in the ring does not affect toxicity in the same way it promotes retention in the liver (Section 1.5). The very limited data available suggest that the PCDFs conform to these rules. There are also parallels established for the PCDDs, with some limited supporting data for the PCDFs, between the acute toxicity of congeners and

a) their potency in inducing aryl hydrocarbon hydroxylase (AHH) activity,

b) the coincident genetic segregation of sensitivity with AHH inducibility,

c) binding affinity of congeners to a cytoplasmic receptor protein.

However, the above are parallels, not rules, and the data for the PCDFs are extremely limited.

Two important issues must be resolved. The first involves the determination or prediction of the relative toxicity of the congeners so that residue data may be interpreted. The second involves the determination of how human sensitivity compares with that of other mammals so that reliable extrapolations can be made. The effects of PCDFs on humans seem generally to parallel those observed in laboratory mammals. However, there are many confounding factors in the human data and the relative sensitivity of humans cannot be gauged from current data.

The lowest observed-effect levels (LOELs) for various toxicological effects for 2,3,7,8-T_4CDF are summarized in Table 1-1. The data are available for several species. The most clearly recognized effects are acute lethality, weight-loss and acne. Although the teratogenic and cancer-promoting potential of 2,3,7,8-T_4CDD has been noted, no data are available for the PCDFs. Extrapolating from

Table 1-1. Reported lowest observed-effect levels (μg.kg-BW^{-1}) for 2,3,7,8-T_4CDF (see Chapter 7).

Effect	Monkey	Rat	Guinea Pig	Mouse
Weight	$500^{\alpha,\delta}$; $0.5^{\beta,\varepsilon}$		$5^{\alpha,\delta}$	
Biochemical	$500^{\alpha,\delta}$; $0.5^{\beta,\varepsilon}$			$300^{\beta,\delta}$
Pathological/Histological	$500^{\alpha,\delta}$; $0.5^{\beta,\varepsilon}$	$50^{\beta,\varepsilon}$	$5^{\alpha,\delta}$	$300^{\beta,\delta}$
Reproductive	NO DATA AVAILABLE			
Teratogenic	NO DATA AVAILABLE			
Carcinogenic (or cancer-promoting)	NO DATA AVAILABLE			
Immunotoxic$^{\zeta}$	$500^{\alpha,\delta}$; $0.5^{\beta,\varepsilon}$		$0.5^{\alpha,\delta}$	$180^{\alpha,\gamma}$; $100^{\beta,\delta}$
LD_{50}	$1000^{\alpha,\delta}$		$>5^{\alpha,\delta}$, $<10^{\alpha,\delta}$	

$^{\alpha}$ Single dose.

$^{\beta}$ Acute (< 3 months) dose.

$^{\gamma}$ i.p.

$^{\delta}$ Gavage

$^{\varepsilon}$ Converted from μg.kg-diet^{-1}.

$^{\zeta}$ Including spleen or thymus atrophy or bone marrow hypoplasia.

2,3,7,8-T_4CDD, the no observed-effect level (NOEL) for cancer or reproductive effects would be expected to be about 1 ng.kg-BW^{-1}.d^{-1} for the more toxic furan congeners. The cost of establishing the actual carcinogenic and reproductive-effect potential of furan congeners would be high. Similarly, it would be expensive to establish data for a wider spectrum of dioxin congeners. The alternative is to improve our understanding of mechanisms, including possible multiple mechanisms, and hence improve our confidence in extrapolations between compounds and congeners.

1.6 EXPOSURE (discussed in detail in Chapter 5)

On the basis of the 1 ng.kg-BW^{-1}.d^{-1} NOEL for 2,3,7,8-T_4CDD reproductive or carcinogenic effects, the NRCC panel (1981a) concluded that if fish were the only contaminated portion of the diet, were eaten only once a week and only 2,3,7,8-T_4CDD was of concern, then a dangerous contamination level in fish would be 3-30 ng.kg^{-1}. This is just at the limits of detection!

Similar calculations cannot be made for the PCDFs because of the lack of toxicology data. However, levels have been measured in fish that greatly exceed the above limits. If humans have a relatively low sensitivity, these residues in food may not present as serious a problem as the PCDD extrapolation indicates. It has already been recommended that research effort be directed towards determining the sensitivity of humans relative to that of laboratory animals, and ranking the relative importance of different sources for human exposure.

Certain occupational groups could be at relatively high risk. These could include workers involved in the manufacture and use of CPs, phenoxy herbicide applicators, and electrical workers. Epidemiology studies for high-risk groups might be useful. Such groups could be identified after the exposure of specific populations was ranked by source, as already discussed.

The biological significance of PCDFs (and PCDDs) produced by incinerators is hard to assess. The bioavailability may be reduced as a result of linkage to the soot-matrix. Inhalation or the consumption of food produced in the vicinity of incinerators could be exposure routes.

1.7 RECOMMENDATIONS

The recommendations identified for the PCDDs in NRCC (1981a, b; Appendices VI and VII) generally apply to the PCDFs. Because of their structural and toxicological similarity to the PCDDs, PCDFs should be treated with the same degree of caution. Research on PCDDs and PCDFs is usually complimentary and the compounds should generally be considered together. Current and proposed control actions for the PCDDs

should be reviewed for their application to the PCDFs. The following specific recommendations are all considered of high priority but are listed in what the authors consider the order of priority:

(1) IDENTIFY ALL CANADIAN SOURCES WITH POTENTIAL FOR RELATIVELY HIGH HUMAN EXPOSURE TO PCDFs (OR PCDDs).
This would involve:

a) DEVELOPING ASSAYS FOR THE BIOLOGICAL ACTIVITY AND BIOAVAILABILITY OF CONGENER MIXTURES PRESENT IN ENVIRONMENTAL SAMPLES
b) COMPILING AN INVENTORY OF CANADIAN SOURCES RATED FOR RELATIVE IMPORTANCE IN TERMS OF THE TOXICITY OF THE CONGENER MIX PRESENT.
c) ASSESSING HUMAN INTAKE RELATIVE TO SOURCES

(2) TOXICOLOGY RESEARCH SHOULD FOCUS ON ESTABLISHING MECHANISMS OF ACTION SO THAT THE CONFIDENCE IN ESTIMATING THE TOXICITY OF ISOMER GROUPS CAN BE IMPROVED.

It is also recommended that:

(3) EPIDEMIOLOGY AND DETAILED EXPOSURE STUDIES BE CARRIED OUT WITH IDENTIFIED HIGH-EXPOSURE GROUPS, e.g. electrical industry workers. This might allow some estimation of the sensitivity of humans relative to that of laboratory mammals.

2.0 SOURCES AND DISTRIBUTION OF PCDFs

2.1 CHEMICAL SOURCES

PCDFs are known to be contaminants of several classes of presently or previously used industrial and agricultural chemicals, including polychlorinated biphenyls (PCBs), chlorinated phenols (CPs), chlorinated phenoxy acids and polychlorinated benzenes (PCBzs).

Because of weaknesses in analytical methodology (Chapter 3.0; NRCC 1981b), the occurrence of PCDFs in chemicals can be described only semi-quantitatively. Specifically, there is a general lack of information on the levels of recovery achieved (residue extraction efficiency) (NRCC 1981b) so that the reported absence of PCDFs in a sample tested often does not preclude their presence even at the stated detection limits. This is particularly true in earlier studies where detection limits were high. False "positive" samples are also quite possible as other organics can mimic the PCDFs (see Chapter 3). Inadequate sampling replicates or controls can reduce the confidence of any study. The level of confidence required depends on the end use. For regulatory purposes confirmation is desirable, but less rigid requirements may be adequate for surveys.

Another problem results from the extreme variation in toxicity of different PCDF congeners (Chapter 7.0). Generally, all of the furans are treated as a group and very few studies report on the level or even the presence of particular congeners in contaminated samples. This is partly due to the lack of sufficient quantities of reference compounds for analysis and the other problems associated with isomer-specific analysis (Chapter 2.0; NRCC 1981b). Noncongener-specific data cannot be related to toxicity nor hence to risk.

2.1.1 POLYCHLORINATED BIPHENYLS (PCBs)

PCBs are a well characterized source of PCDFs. Although PCB use has been restricted mainly to closed systems since about 1970[α], and their manufacture and distribution in North America ceased totally in 1977[α], the higher chlorinated residual PCBs are extremely persistent in the environment, and these compounds are still of concern. The Monsanto Chemical Company produced an estimated 0.65 million tonnes between 1930 and 1975, about 87% of which was marketed domestically and about 67% of which was exported to Canada (Brinkman and DeKok 1980). Large quantities of PCBs have entered the environment and environmental

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[α] Monsanto, the major manufacturer and distributor of PCBs in North America, voluntarily restricted their open use in 1970-1971 and withdrew from the manufacture and distribution of PCBs by October 1977 (Roberts et al. 1978). In Canada, PCBs have been used only in closed systems (transformers, light ballasts, etc.) since 1972 (Environmental Contaminants Committee 1976).

contamination is still occurring because of the disposal and repair of articles containing PCB. Commercial PCB preparations have been used in lubricants, dielectric fluids, heat transfer agents, protective coatings for wood, metal or concrete, etc. Many of these materials may still be in use, or have been disposed of using methodologies that permit leaks to the environment. PCBs in older electrical equipment, which may have been exposed to high temperature over a number of years, are of particular concern. In 1982, at least 16 000 tonnes of PCBs were in use in Canadian electrical equipment, at least 19 tonnes in mechanical equipment and at least 997 tonnes were in storage (Environment Canada 1983a). PCBs have been prohibited from use in all products and equipment (except electrical) manufactured in or imported to Canada after September 1977, and in equipment manufactured or imported after July 1980 (Environmental Contaminants Act 1980).

PCDFs were first reported to be contaminants of German and French PCBs in 1970 (Vos et al. 1970). Since then their presence has been confirmed in commercial samples of PCB produced in all countries examined (Table 2-1). Multiple isomers of di through hepta congeners have been identified. American samples of commercial Aroclor have been reported to contain from < 1 to 16 mg-PCDF.kg-PCB-sample^{-1} (Table 2-1). In the sample contaminated with 16 mg-PCDF.kg^{-1}, 2.4 mg.kg^{-1} was tentatively identified as 2,3,7,8-T_4CDF (Morita et al. 1977). An American sample of 99% pure commercial hexachlorobiphenyl (2,4,5,2',4',5'-H_6CB) was reported to contain a total of 48.4 mg-PCDF.kg-PCB^{-1}, including 44 mg.kg^{-1} identified as 2,3,7,8-T_4CDF (Goldstein et al. 1978). PCB samples from Japan and Europe were generally quite contaminated with PCDFs; Japanese commercial Kaneclor samples reportedly contained from 1.1 to 33 mg-PCDF.kg^{-1}, French Phenoclor samples contained from 3.8 to 13 mg-PCDF.kg^{-1} and German Clophen samples from 4.9 to 17 mg-PCDF.kg^{-1} (Table 2-1).

In PCB samples which have been subjected to heat, much higher PCDF levels have been reported. There is often confusion on the meaning of the terms pyrolyze, burn or combust. The last two terms generally denote a chemical process that produces light and heat while the former usually indicates irreversible chemical decomposition caused by heat (Kaplan et al. 1983). For the data reviewed here, we will not attempt to clarify whether PCBs are actually combusted under specified conditions, but will use the term if the authors do. Since PCBs are known to be quite resistant to burning, it seems unlikely that combustion would occur without the use of supplementary fuel.

Miyata and Kashimoto (1976) heated samples of Kaneclor KC400 to various temperatures up to 360°C for 2 to 20 days (Table 2-2). While the quantities of PCDF formed tended to increase with thermal exposure (heating time and temperature), no general trends were apparent. They found that the presence of stainless steel or water (but not both) enhanced PCDF formation. Morita et al. (1978) found that when PCBs were heated at various temperatures for one week or more in the presence of oxygen, PCDFs were formed at temperatures over 270°C and reached maximum levels at about 300°C. They speculated that some decomposition of PCDF

Table 2-1. PCDFs as contaminants of PCB formulations.

Country	Product (lot and/or production year)	Isomer ($mg.kg^{-1}$)[a] 2-Cl	3-Cl	4-Cl	5-Cl	6-Cl	7-Cl	Total	Total Isomers detected	Reference
USA	Aroclor 1248 (1969)	NA[b]	NA	0.5 (0.33; 2,3,7,8)	1.2 (0.83; 2,3,7,8)	0.3		2.0	10-12	Bowes et al. (1975a, b)
"	"		2 isomers	3 isomers	4 isomers			2.0	11	Miyata and Kashimoto (1976)
"	"		ND[c]	0.5 (0.2; 2,3,7,8)	2-3	ND		2.8		Morita et al. (1977)
"	Aroclor 1260 (AK3)							< 1		Vos et al. (1970)
"	" (AK3)	NA	NA	0.2	0.3	0.3	NA	0.8	10-12	Bowes et al. (1975a, b)
"	" (1969)	NA	NA	0.1	0.4	0.5	NA	1.0	10-12	
"	"		1 isomer	3 isomers	3 isomers	3 isomers	1 isomer	2.0	11	Miyata and Kashimoto (1976)
"	"		ND	0.8 (<; 2,3,7,8)	0.9	0.5		2.2		Morita et al. (1977)
"	Aroclor 1254 (1969)	NA	NA	0.1 (0.1; 2,3,7,8)	0.2 (0.12; 2,3,4,7,8)	1.4	NA	1.2	10-12	Bowes et al. (1975a, b)
"	"		1 isomer	4 isomers	6 isomers	5 isomers		9.0	16	Miyata and Kashimoto (1978)
"	" (1970)	NA	NA	0.2	0.4	0.9	NA	1.5	10-12	Bowes et al. (1975a, b)
"	"		ND	0.1	3.6	1.9		5.6		Morita et al. (1977)
"	"	NA	0.1	0.25	0.7	0.81		1.9		Rappe and Buser (1981)
"	" (KK602)	NA		0.05	0.1	0.02		0.2		Rappe and Buser (1981)
"	Aroclor 1016 (1972)	NA	NA	< 0.001						Bowes et al. (1975a, b)
"	Aroclor 1242			2.3 (0.2; 2,3,7,8)	2.2			4.5		Morita et al. (1977)
"	"		3 isomers	5 isomers	3 isomers			3.0		Miyata and Kashimoto (1978)
"	Aroclor 1264			4.8 (2.4; 2,3,7,8)	9.4	2.0		16.2		Morita et al. (1977)
"	Aroclor 241			2.4 (1.1; 2,3,7,8)	2.7	0.8		5.9		Morita et al. (1977)
"	99% pure commercial 2,4,5,2',4',5' HCB	NA	4.2 (1.8; 2,3,8)	2 (44; 2,3,7,8)	0.2	<0.05	NA	48.4		Goldstein et al. (1978)
"	"Pure HCB" (?)[d]	NA	<0.05	(0.05; 2,3,7,8)	<0.05	<0.095	NA	>0.05		"
Japan	Kaneclor 200			(0.1; 2,3,7,8)	(0.1; 2,3,7,8)			?		Bowes et al. (1975a, b)
"	Kaneclor 300			6.7 (2.2; 2,3,7,8)	1.6			8.3		Morita et al. (1977)
"	" (<1972)		2 isomers	8 isomers	6 isomers	5 isomers		9	2	Miyata et al. (1976)
	" (<1968)			1 isomers	1 isomers			1	2	Nagayama et al. (1975)
"	Kaneclor 400		0.3	12.2 (1.6; (2,3,7,8)	10.4	0.9		23.8		Morita et al. (1977)
"	"		2.3	26.2	2.4	1.9		33.0		Miyata et al. (1976)
		3 isomers	5 isomers	8 isomers	5 isomers	3 isomers				Miyata and Kashimoto (1978)
"	" (<1968)	3 isomers	5 isomers	5 isomers	3 isomers			18	14	Nagayama et al. (1975)
"	Kaneclor 500			? (0.19; 2,3,7,8)	2 (0.08; (2,3,4,7,8)			?		Bowes et al. (1975a)
"	"		0.2	1.7	1.1	3.1		6.1		Morita et al. (1977)
"	" (<1972)		1 isomer	4 isomers	3 isomers	4 isomers	2 isomers	4	14	Miyata et al. (1976)
"	" (<1968)				1 isomers	3 isomers	1 isomers	4	5	Nagayama et al. (1975)
"	Kaneclor 600			0.2 (0.1; (2,3,7,8)	0.5	0.4		1.1		Morita et al. (1977)
"	" (<1972)			1 isomer	1 isomer	2 isomers	2 isomers	5	6	Nagayama et al. (1975)
"	" (<1972)			5 isomers	5 isomers	4 isomers	2 isomers	4	16	Miyata et al. (1976)

Table 2-1. (CONT'D)

Country	Product (lot and/or production year)	Isomer ($mg.kg^{-1}$)[α] 2-Cl	3-Cl	4-Cl	5-Cl	6-Cl	7-Cl	8-Cl	Total	Total Isomers detected	Reference
Germany	Clophen A60 (912436)	<1	<1	>1	>1, <5	<1	<1	<1	>2, <5		Vos et al. (1970)
"	"	NA	NA	1.4	5.0	2.2	NA	NA	8.6	10-12	Bowes et al. (1975a, b)
"	Clophen A30		1.6	2.3 (1.6; 2,3,7,8)	1.0				4.9		Morita et al. (1977)
"	"		3 isomers	5 isomers	4 isomers				6.0	12	
"	Clophen A40		1.5	5.4 (2.1; 2,3,7,8)	6.9				13.8		Morita et al. (1977)
"	"		4 isomers	5 isomers	5 isomers				5.0	14	Miyata and Kashimoto (1976)
"	Clophen A50		1.7	8.3 (3.6; (2,3,7,8)	4.1	1.8			15.9		Morita et al. (1977)
"	"	2 isomers	5 isomers	6 isomers	6 isomers	3 isomers			17.0	22	Miyata and Kashimoto (1976)
France	Phenoclor DP-6	<1	<1	>1	>1, <20	<1	<1	<1	>2, <20		Vos et al. (1970)
"	"	NA	NA	0.7	10.0	2.9	NA	NA	11.6	10-12	Bowes et al. (1975b)
"	"		0.2	2.1 (0.9; 2,3,7,8)	2.6	5.6			10.5		Morita et al. (1977)
"	"			4 isomers	5 isomers	5 isomers	3 isomers		13.0	17	Miyata and Kashimoto (1976)
"	Phenclor DP-4			1.7 (0.7; 2,3,7,8)	1.6	0.5			3.8		Morita et al. (1977)
"	"		3 isomers	5 isomers	5 isomers				8.0	13	Miyata and Kashimoto (1976)
"	Phenoclor DP-5			4.6 (2.2; (2,3,7,8)	2.7	2.6			9.9		Morita et al. (1977)
"	"			4 isomers	4 isomers	1 isomer	2 isomers		11.0	11	Miyata and Kashimoto (1976)

[α] Total isomer level with identified congener levels in brackets and total number of isomers specified.

[β] Tentative; identifier not detected.

[γ] ND = not detected; detection limit not known

[δ] NA = not analyzed

Table 2-2. PCDFs in Kaneclor KC400 formulations subjected to heat (after Miyata and Kashimoto 1978).

Temperature (°C)	Time (d)	"Catalyst"	mg.kg^{-1}				
			3 Cl	4 Cl	5 Cl	6 Cl	Total
Not heated	-	-	2.3	26.2	2.4	1.9	33
180	5	-	3.6	33.8	2.7	1	41
250	5	-	3.1	32.9	2.5	1	40
360	2	-	15.2	64.9	3.9	0	84
360	10	-	24.1	65.1	4.8	0	94
360	20	-	11.1	52.1	1.8	0	65
360	2	Stainless steel	33	127	25.7	2.7	188
360	5	"	42.3	121.2	16.3	2.2	182
360	10	"	48.1	133.4	20.0	3.5	205
360	20	"	42.9	32.7	1.5	0	77
360	2	Water	42.8	152.1	25.7	0	222.6
360	20	"	43.1	152.4	16.6	0	152.4
360	2	Stainless steel and water	10.7	82.3	8.4	0	101.4
360	20	"	14.7	46.6	9.9	0	71.2

might be occurring at 330°C. They also found that maximum levels of PCDFs were reached after heating the PCBs for one week in O_2 or 2 weeks in air. They postulated that there is a critical point for heating conditions as PCDFs are also subject to thermal decomposition. Under their optimum conditions PCDFs were formed to concentrations about 2000 mg per kg of starting material (0.2%). In the one sample analyzed for isomers, which contained an unidentified amount of PCDF, the level of 2,3,7,8-T_4CDF was 80 $mg.kg^{-1}$.

Buser et al. (1978d) found that at least 50 times as much PCDFs (and up to 16×10^3 $mg.kg^{-1}$) were formed when H_6CB isomers were heated at 550°C than at 700°C. At 700°C, levels were below 10 $mg.kg^{-1}$.

Pyrolysis of PCBs can lead to the formation of very high levels of PCDFs. Data on some PCDF levels in heated or pyrolyzed PCBs are summarized in Table 2-3.

Buser and Rappe (1979) pyrolyzed individual PCB isomers (98% pure), initially containing 0.01% PCDFs, at 600°C in quartz mini-ampoules. They found that PCDFs were formed via intramolecular cyclizations (with loss of ortho Cl_2 or HCl) giving yields in the range of 0.1% to several percent. The PCDFs appeared to be formed via four previously speculated general reaction routes and the authors thus found it possible to predict the structure of the resulting PCDFs. In most cases they were able to tentatively identify the predicted reaction products and found good agreement between the analyzed and predicted results.

The formation of PCDFs from the pyrolysis of PCBs is of environmental concern. PCBs may be disposed of by incineration or exposed to very high temperatures during accidental building fires (see Section 2.1.1.2). Rappe et al. (1983a) reported that no detectable PCDDs or PCDFs were formed when PCBs were destroyed in a rotary cement kiln at 1450 to 2000°C, which suggests this may be a relatively safe method for PCB destruction.

2.1.1.1 Electrical Equipment

In 1982, the number of PCB electrical units still in service in Canada exceeded 204 400; they contained in excess of 16 000 tonnes of PCBs (Environment Canada 1983a). The conversion of PCBs to PCDFs in electrical equipment is also of concern.

Chittim et al. (1979) detected and quantified PCDFs in Canadian electrical transformers containing PCBs. They reported that the concentration of 2,3,7,8-T_4CDF in transformer askarels varied from 0.005 to 5.0 $mg.kg^{-1}$ and tended to increase with the length of time the askarel had been in service when other variables were held constant (Table 2-4). However, their analysis used gas chromatography with a packed column that provides relatively poor resolution, and what was

Table 2-3. PCDFs in heated or pyrolyzed PCBs.

Starting Material	Heating Conditions	Homologues ($mg.kg^{-1}$)	Yield (mg.kg-starting material^{-1})	Reference
Aroclor 1254		Mono through penta homologues. Major P_5CDFs were 1,3,4,7,9- and 1,3,4,7,8[a]-; also 1,2,3,7,8- and 2,3,4,7,8-. Major T_4CDF was 2,3,7,8-	2×10^4	Buser et al. (1978a)
Aroclor 1260		Tri through octa homologues. Major components as above except 1,3,4,7,9-	2×10^4	"
2,4,5,2',4',5'-H_6CB	air, 550°C	2,3,7,8-T_4CDF (1.6×10^4); 2,3,4,7,8-P_5CDF; and 1,3,4,7,8-P_5CDF (2×10^3)	1.8×10^4	"
2,4,6,2',4',6'-H_6CB	air, 550°C	1,3,7,9-T_4CDF (5×10^3)[a]; 1,3,4,7,9-P_5CDF (5×10^3)	6×10^3	Buser et al (1978d)
Individual PCB Isomers	air, 600°C	Isomer pattern depended on starting material	~10^3 to 10^4	Buser and Rappe (1979)
Aroclor 1248	air, 300°C, 1 wk		10	Morita et al. (1978)
"	O_2, 300°C, 1 wk		2000	"
"	N_2, 300°C, 1 wk		2	"
Aroclor 1254 500-650°C	air	T_4CDF	141-197	Chittim et al. (1979)
Aroclor 1016	"	T_4CDF	2.4-2.6	"
Pyranol	"	T_4CDF	424-489	"
Interteen	"	T_4CDF	146-240	"
Chlorextol	"	T_4CDF	194-248	"
PBB fire retardant	O_2 400°C	2,3,7,8-T_4CDF (40) P_5CDF (4)	44	O'Keefe (1978)
"	N_2 400°C		0	"

[a] Isomer expected but no confirmation.

Table 2-4. PCDFs in transformer askarels; variation with time in service[α] (after Chittim et al. 1979).

Year of installation	Aroclor	Chlorobenzenes/ PCBs (%)	T_4CDF (mg.kg-askarel^{-1})
1957	1260	51/49	0.780
1961	1260	53.6/46.4	1.110
1963	1260	50.6/49.4	1.020
1963	1260	52.4/47.6	0.790
1963	1260	38.6/61.4	0.350
1965	1260	37.2/62.8	0.290
1970	1254	29.6/70.4	0.550
1970	1254	28.0/72.0	0.470
1971	1254	29.0/71.0	0.290
1974	1254	28.4/71.6	0.051
"New"	1254	30.6/69.4	0.041

[α] The only variable was "time in service". All these transformers were 1500 kV. Volume ranged from 1151 to 2093 L.

reported as "2,3,7,8-T_4CDF" may actually have included other tetra isomers. P_5CDFs and H_6CDFs were also detected. The conclusions may be further confounded by other compounds that may coelute with the PCDFs. When only the kV/volume ratio was varied, the T_4CDF levels showed no observable trend. The three major fluid types (Pyranol, Inerteen and Chlorextol) showed some variations in PCDF levels but it is not clear whether this was due to differences in the fluid (e.g. in the antioxidants used) or to transformer construction. If an O_2 source is necessary for the generation of PCDFs during sparking, this could be supplied by the epoxides generally present in transformers. Periods of abnormal operation (e.g. arcing) apparently did not affect PCDF concentrations. The highest concentrations of T_4CDF (2.2 and 4.7 $mg.kg^{-1}$) were found in old transformers in service since 1947. It is possible that this, as well as the reported correlation between PCDF concentrations and length of time in use, may simply reflect a historical contamination pattern. Upon analysis by high resolution GC/MS, "used" askarel samples (1938-1940) from the Canadian General Electric Company were reported to contain 0.6 to 1.86 µg-PCDFs.L^{-1} (NEMA 1982). Miyata and Kashimoto (1978) measured much higher PCDF levels (over 500 $mg.kg^{-1}$) in a Kaneclor-based transformer fluid that had been in use for several years. Tri through hexa homologues were detected.

Many mineral-oil filled tranformers, in use in Canada, are also contaminated with PCB at levels above 50 $\mu g.L^{-1}$ (Czerkawski *et al.* 1983).

2.1.1.2 Building and Electrical Equipment Fires

Building fires involving electrical equipment containing PCBs or PCBzs are known to generate PCDFs and other chlorinated organics (Czerkawski *et al.* 1983). Transformers, capacitors, light ballasts, etc. may be of concern. Generally, attention is focused on the cleanup of the building to a condition considered safe for rehabitation. Since the contaminants generally occur in the soot and the thickness of the soot layer is quite variable, concentrations can be more informative when expressed per square metre of building space rather than per mg soot.

Rappe *et al.* (1982, 1983a) analyzed soot samples from a capacitor battery fire in Stockholm. A series of capacitors filled with a French PCB equivalent to Aroclor 1242 had been ignited (11 kV arc), presumably because of a malfunction in the capacitor battery. Wipe tests taken about 1 m from the capacitors were analyzed. Mainly tetra (1.6 $\mu g.m^{-2}$) and penta (0.17 $\mu g.m^{-2}$) PCDFs were present. The contaminants reported were mainly diphenylamines; no detectable PCDDs were formed. Among the PCDFs, the highly toxic 2,3,7,8-T_4CDF and 2,3,4,7,8-P_5CDF isomers were identified as major components (Table 2-5).

Rappe *et al.* (1982, 1983a) also reported the presence of PCDFs after a fire in a 400 V capacitor battery, containing PCB and mineral

Table 2-5. PCDFs in samples from a capacitor battery fire in Stockholm (after Rappe et al. 1983a)

PCDF	ng.m^{-2}
2,3,7,8-	150
1,2,7,8-	150
2,3,6,8-	125
1,4,6,9-	75
2,4,6,7-	37
3,4,6,7-	7.5
1,3,6,7- + 1,3,6,9-	300
other T_4CDFs	750
$\sum T_4$CDFs	1600
2,3,4,7,8-	45
1,2,4,7,8-	41.8
1,2,3,6,7-	18.8
1,3,4,7,8-	11
2,3,4,6,7-	7.5
1,2,4,6,8-	3.8
1,2,4,8,9-	3.8
2,3,4,6,8-	2
1,2,3,7,8- + 1,2,3,4,8-	15
other P_5CDFs	19
$\sum P_5$CDFs	170
$\sum H_6$CDFs	< 0.5
$\sum$PCDFs	> 1775

oil, that fed a high frequency oven in a metal treatment factory in Sweden. About 2 h elapsed before the fire was completely extinguished. They analyzed soot from several locations (Table 2-6) and found high PCDF residues, including the 2,3,7,8- isomer. In this case, dichloropyrenes were apparently[α] the main contaminant with PCDDs undetectable.

Janson and Sundström (1982) measured PCDF levels in capacitors and in environmental samples (Table 2-7) after a fire in a transformer station north of Stockholm. Eighteen capacitors (100 kV each) were "burned". 2,3-D_2CDF, 2,3,8-T_3CDF and 2,3,7,8-T_4CDF were tentatively identified among the many isomers present in the liquid from the exploded capacitors.

In 1981, a transformer explosion in an office building in Binghamton, N.Y. spilled about 800 L of askarel solution and resulted in a layer of contaminated soot on virtually all surfaces in the building (Schecter 1983). The transformer was filled with Pyranol containing 65% Aroclor 1254 and 35% chlorinated benzenes.

Smith et al. (1982) analyzed soot samples taken from the third- to fourth-floor stairwell after the Binghamton fire and measured 120 to 270 mg.kg^{-1} 2,3,7,8-T_4CDF (cf. ~3 mg.kg^{-1} 2,3,7,8-T_4CDD). Total PCDF content of the soot was estimated in the order of 0.5%. Rappe et al. (1982; 1983a) reported that the major PCDF isomers included: 2,3,7,8- among the tetra homologues; 1,3,4,7,8-, 1,2,3,7,8- and 2,3,4,7,8- among the penta homologues; and 1,2,3,4,7,8- and 1,2,3,6,7,8- among the hexa homologues. Rappe et al. (1983a) also reported PCDDs to be present, with 2,3,7,8-T_4CDD and 1,2,3,7,8-P_5CDD dominating. PCDDs have been reported only in fires involving chlorobenzenes.

The time lapse between a fire and the clean-up operation could be an important variable in determining the success of the cleanup and the levels of contaminants measured. This is because lipophilic organics including the PCDFs might be readily absorbed into the fabric of the building, such as the paint on walls or varnish on floors. Not only would the PCDFs then be hard to remove but also the levels may not show up in swab tests.

2.1.1.3 Wire Reclamation

Incineration is a commonly used method of recovery of metals from scrap electrical cable; the combustible portion of the cable can be composed of a wide variety of materials, and older cables can contain PCBs (Hryhorczuk et al. 1981).

[α] No final proof due to lack of standards.

Table 2-6. PCDF residues after a capacitor fire in a metal treatment factory (Rappe et al. 1982).

Homologue	Sample[α] ($ng.m^{-2}$) 1	2	3	4
$\sum T_4CDF$	100	600	< 1	ND[γ]
2,3,7,8-TCDF	20	100	< 1	10[β]
$\sum P_5CDF$	40	100	ND	ND
$\sum H_6CDF$	40	60	ND	ND
$\sum H_7CDF$	8	8	ND	ND
$\sum O_8CDF$	5	5	ND	ND

α Two samples at each location:
1. On the floor, in the capacitor battery
2. On the floor, close to the capacitor battery
3. On the wall in the capacitor room, 2 m from the battery and 3 m above the floor
4. On a bench 10 m from the oven, on the ground floor

β Different isomers than those found in samples 1 and 2.

γ Detection limit unspecified.

Table 2-7. PCB and PCDF levels in capacitors and in environmental samples after a transformer station fire (after Jansson and Sundström 1982).

	mg.kg-sample^{-1}					
	Aroclor 1242	M_1CDF	D_2CDF	T_3CDF	T_4CDF	$\sum PCDF$
Liquid in intact capacitor	$0.83\text{-}0.86\times10^6$	0.2-0.5	0.2	0.2-0.3	0.2-0.3	0.9-1.1
Liquid in exploded capacitor	0.93×10^6	24	38	11	3	75
Liquid on exploded capacitor	$0.49\text{-}0.60\times10^6$	12-21	9-21	3-7	2	27-52
Extract from wire fence	0.3×10^3	<0.03	<0.2	<0.03	<0.04	<0.3
Pine needles	0.3	ND	ND	ND	ND	ND

Hryhorczuk et al. (1981) analyzed furnace and fly ash from a wire-reclamation incinerator, soil from the area, and horse fat and liver samples for PCDFs after an outbreak of "unusual illnesses" in humans and animals residing within 1.3 km of the incinerator, located in a rural, midwestern U.S. community. All of the observed symptoms and signs more closely resembled those associated with smoke inhalation than those of PCDF or PCDD exposure.

The wire-reclamation facility incinerated wire, cable and X-ray film; about 45-82 tons of scrap were incinerated per month. Because of improper operation and a consequent smoke problem, the facility was closed by temporary injunction in April, 1980. The levels of T_4CDD and T_4CDF measured in fly and furnace ash, soil and horse tissue are presented in Table 2-8. The significance of the data is difficult to assess as tissue control samples were not measured.

2.1.2 CHLOROPHENOLS AND RELATED PRODUCTS

Chlorophenols (CPs) have been in use since the 1930s, principally as biocides (such as fungicides) (e.g. 2,4,6-T_3CP, 2,3,4,6-T_4CP and PCP are used as wood preservatives), bactericides (e.g. in industrial oils) or slimicides. 2,4-D and 2,4,5-T are also precursors for a variety of phenoxy acid herbicides. The production methods and uses of CPs have been reviewed (NRCC 1982).

While CPs are toxic chemicals per se (NRCC 1982), it is their contamination with PCDFs that is the concern of this document. Commercial CP samples were first reported to be contaminated with PCDFs by Firestone et al. (1972). As would be expected, the congener pattern varied with the chlorination pattern of the CPs. Since then, PCDFs have been reported to occur at different levels (up to hundreds of $mg.kg^{-1}$) in a variety of chlorophenols and derivatives (Tables 2-9).

Improved process control has tended to decrease the levels of PCDDs in CPs in recent years (Lindahl et al. 1980). However, it is now known that PCDDs can be photochemically or thermally generated from less toxic contaminants such as the polychlorodiphenylethers (Norström et al. 1976, 1977; Lindahl et al. 1980). This raises the question of whether PCDFs could also be generated and whether such reactions could be of environmental significance.

PCDFs have also been reported to occur at higher levels in a variety of pyrolyzed CP products. Taylor et al. (1983) analyzed PCP-contaminated wood samples and the ash from an industrial incinerator burning liquid and solid wastes that contained PCP. Their data (Table 2-10) suggest that when PCP-containing materials are incinerated, the concentrations and number of isomers of the lower Cl homologues are enhanced relative to the higher Cl homologues. This suggests a thermal degradation of the higher chlorinated PCDDs and PCDFs, which may be accompanied by concurrent generation of the lower Cl homologues.

Table 2-8. Levels of T_4CDD and T_4CDF associated with a wire-reclamation incinerator[α] (after Hryhorczuk et al. 1981).

Sample	µg.kg^{-1}	
	T_4CDD	T_4CDF
Soil: 5 cm topsoil from 50 m east of stack	21	230
Soil: Core sample (depth 20 cm) from 1 km NE of incinerator (this serves as control sample)	ND (< 3)	ND (< 3)
Furnace Ash	58	730
Fly Ash	410	11 600
Horse fat[β]	45	165
Horse liver[β]	ND (< 6)	57

[α] Analysis was not congener specific.

[β] Control samples were not measured.

Table 2-9. PCDFs in commercial chlorophenols.

Product[α]	Homologue	Concentration ($mg.kg^{-1}$)	Reference
2-C_1P	T_4CDF	?[β]	Firestone et al. (1972)
2,4,5-T_3CP-Na	T_3CDF, T_4CDF, P_5CDF	"	"
2,4,5-T_3CP	T_3CDF, T_4CDF, P_5CDF, H_6CDF	"	"
2,3,4,6-T_4CP	T_4CDF, P_5CDF, H_6CDF, H_7CDF. P_8CDF	"	"
P_5CP-Na	P_5CDF, H_6CDF, H_7CDF	"	"
P_5CP	T_4CDF, P_5CDF, H_6CDF, H_7CDF, O_8CDF	"	"
Technical P_5CP (< 1960)	H_6CDF, O_8CDF	?[β]	Plimmer et al. (1973)
Dowicide-6 (mainly T_3CP)	T_4CDF	0.2	Buser (1975)
	P_5CDF	0.2	"
	H_6CDF	230	"
	H_7CDF	500	"
	O_8CDF	135	"
	Total PCDFs	865	
Dowicide-7 (mainly P_5CP)	T_4CDF	< 0.2	"
	P_5CDF	< 0.2	"
	H_6CDF	39	"
	H_7CDF	280	"
	O_8CDF	230	"
	Total PCDFs	549	
2,4,6-T_3CP (Scandinavia)	T_4CDF (major components) 2,3,7,8-, 2,3,6,8-, 2,4,6,8,-)	1.5	Rappe et al. (1978a)
	P_5CDF (1,2,4,6,8- dominating also 2,3,4,6,8-, 2,3,4,7,8-, 1,3,4,7,8-, 1,2,3,7,8-)	17.5	"

Table 2-9. (CONT'D)

Product	Homologue	Concentration ($mg.kg^{-1}$)	Reference
	H_6CDF (1,2,3,4,6,8- dominating also 1,2,4,6,7,8- and 1,2,4,6,8,9-)	36.0	Rappe et al. (1978a)
	H_7CDF (including 1,2,3,4,6,7,8- and 1,2,3,4,6,8,9-)	4.8	"
	Total PCDFs	60.0	"
2,3,4,6-T_4CP (Scandinavia)	T_4CDF	<0.5	"
	P_5CDF (only 1,2,4,7,8- detected)	10.0	"
	H_6CDF (1,2,3,4,6,8- and 1,2,4,6,8,9- dominate, also 1,2,4,6,7,8-)	70.0	"
	H_7CDF (1,2,3,4,6,7,8- and 1,2,3,4,6,8,9- present equally)	70.0	"
	O_8CDF	10.0	"
	Total PCDFs	160.0	"
P_5CP (U.S.A.)	T_4CDF	0.9	"
	P_5CDF (only 1,2,4,6,8- identified)	4.0	"
	H_6CDF (1,2,4,6,8,9- dominates, also 1,2,3,4,6,8- and 1,2,4,6,7,8-)	32.0 120.0	"
	H_7CDF (1,2,3,4,6,8,9- dominates, also 1,2,3,4,6,7,8-) O_8CDF	130.0	"
	Total PCDFs	280.0	"
2,4,6-T_3CP (50% aqueous, Sweden)	P_5CDF and H_6CDF (in 3 samples)	10-100	"

[α] Always mixtures; never pure isomer.

[β] Levels not quantified. Detection limits not stated but likely 0.02 to 0.05 $mg.kg^{-1}$.

Table 2-10. PCDDs and PCDFs in waste feed stock and various effluents resulting from the incineration of PCP containing wastes (after Taylor et al. 1983).

Homologue	4.5% PCP in oil		Sludge Liquid		Ash	
	No. isomers	µg.kg^{-1}	No. isomers	µg.kg^{-1}	No. isomers	µg.kg^{-1}
M_1CDF	4	2	2	0.6	3	75
M_1CDD	2	1.5	1	0.2	1	1
D_2CDF	2	2	ND	ND	8	25
D_2CDD	2	2	ND	ND	4	5
T_3CDF	4	10	ND	ND	8	15
T_3CDD	2	3.5	ND	ND	5	2
T_4CDF	5	18	ND	ND	7	7
T_4CDD	-	1.1	ND	ND	4	3.4
P_5CDF	5	137	1	0.3	5	8
P_5CDD	6	33	3	0.6	5	32
H_6CDF	5	1813	2	0.8	5	5
H_6CDD	4	574	4	2.5	5	81
H_7CDF	2	114	2	13	2	6
H_7CDD	2	256	2	140	2	117
O_8CDF	1	711	1	3.2	1	2
O_8CDD	1	3996	1	450	1	198

Rappe et al. (1983a) analyzed samples of ash from an industrial boiler used to burn PCP. They identified a multitude of PCDDs and PCDFs; total levels were about 5 $mg.kg^{-1}$ and 2.5 $mg.kg^{-1}$, respectively, and significant amounts of congeners were chlorinated in the more toxic positions (see Chapter 7) (Table 2-11). The concentration of T_4-PCDFs and P_5CDFs was as high as that of T_4-PCDDs and P_5CDDs.

2.1.2.1 The Wood Industry

The extensive use of CPs in the wood industry has been reviewed (NRCC 1982). The levels of PCDFs associated with wood industry products internationally are summarized in Table 2-12. PCDFs and PCDDs have also been found in the livers of chickens bedded on contaminated wood shavings (Section 2.4.3). Some restrictions were placed on CP use in 1981 (Agriculture Canada 1980; discussed in NRCC 1982). So far, there is no restriction on CP, PCDD or PCDF levels in animal bedding.

PCP samples from 6 Canadian wood processing plants were all reportedly contaminated with 1.5 to 3 $mg.PCDF.L^{-1}$ (Environment Canada 1982). Since these samples were of a 5% PCP solution, the PCP used presumably contained 30 to 60 $mg\text{-}PCDF.L^{-1}$. All homologues were detected, with hepta and octa being dominant. A variety of effluent samples from these plants were contaminated with 2.6 to 99 $ng\text{-}PCDF.L^{-1}$ (Table 2-13).

2.1.2.2 2,4-D and 2,4,5-T Phenoxy Acid Herbicides

The dioxin problem was originally brought to prominence as a result of spraying herbicides such as Agent Orange, containing 2,4-D and 2,4,5-T, in Vietnam during the 1960s. These herbicides were often found to be contaminated with PCDDs (Table 2-14). Some formulations were found to contain PCDFs also (Table 2-14).

PCDFs detected in some 2,4,5-T and 2,4-D formulations are summarized in Table 2-14. The total PCDF levels reported range from <0.05 to 0.7 $mg.kg\text{-}formulation^{-1}$. In the few congener specific analyses, 2,3,7,8-T_4CDF was not detectable. In these earlier analyses other chlorinated compounds could have been measured as PCDFs or PCDDs (see Chapter 3).

Since the 1970s, Canadian government regulations have set standards for manufacturers to minimize the levels of PCDD in phenoxy herbicides, but little information is available on PCDF levels in today's formulations. Almost no information is available on the levels of either PCDDs or PCDFs prior to the 1960s or on concentrations in those samples used for toxicity studies.

The data in Table 2-14 indicate that the presence or absence of PCDDs is not a consistent indicator of the level of PCDF

Table 2-11. PCDF levels in ash from an incinerator burning PCP (Rappe et al. 1983a).

Homologue	$mg.kg^{-1}$	Ratio PCDF/PCDD
$\sum T_4CDF$	0.9	0.96
2,3,7,8-TCDF	0.1	
$\sum P_5CDF$	1.5	1.05
1,2,3,7,8-PCDF	1.5	
2,3,4,7,8-PCDF	0.1	
$\sum H_6CDF$	0.15	0.14
1,2,3,4,7,8-HCDF	0.02	
$\sum H_7CDF$	?	0.08
$\sum OCDF$	0.006	0.03

Table 2-12. Occurrence of PCDFs in products associated with the wood industry.

Source	PCDFs	Reference
Dust from sawmill using 2,3,4,6 PCP	Same penta and hepta PCDFs as commercial sample (Table 2-9)	Rappe et al. (1978a)
Sludge from dipping tank (Pentachlorophenate)	Two hepta and two octa PCDFs	Jensen and Renberg (1973)
Dust from various locations[α] in a trimming-grading plant, where 2,3,4,6-T_4CP Na salt was used as a spray	In 4 samples, 1-10 $mg.kg^{-1}$ hexa and hepta PCDFs[α]	Levin and Nilsson (1977); Levin et al. (1976)
Dust from various locations in a trimming-grading plant, where 2,3,4,6-T_4CP-Na salt was used as a dip	< 0.5 $mg.kg^{-1}$ in 3 samples	Levin et al. (1976)
Sludge from above dipping tank	700 $mg.kg^{-1}$	"

[α] Levels correspond to concentrations of 2,3,4,6-T_4CP and PCP in samples:

Sample		2,3,4,6-T_4CP ($mg.kg^{-1}$)	P_5CP ($mg.kg^{-1}$)	PCDF ($mg.kg^{-1}$)
(trimming)	1	300	100	6
	2	110	30	1
(grading)	3	100	100	3
	4	800	400	10
(packaging)	5	70		1

Table 2-13. PCDFs in effluents and sludge from Canadian wood processing plants (after Environment Canada 1982).

Plant	Sample[a]	ng.L^{-1}					
		T_4CDF	P_5CDF	H_6CDF	H_7CDF	O_8CDF	Total
1	Effluent flowing into a carbon absorption unit	<0.1	<0.1	0.3	0.3	2.0	2.6
	Effluent flowing out from the carbon unit	<0.1	<0.1	0.1	0.1	8.0	8.2
2	Effluent (settling tank, oil separator) from combined main ditch	0.7	1.5	11.5	47.7	37.1	99.1
	Effluent from ditch further downstream	<0.1	<0.1	0.2	0.3	<0.1	0.5
	Sludge from settling tank (grab sample)	9.5	77.0	11.2	250.0	222.0	570
3	Cooling water condensate and surface runoff (settling tank)	<0.1	<0.1	<0.1	<0.1	1.3	1.3
	Oil contaminated process water	1.1	<0.1	3.2	21.1	39.5	65.0
4	Condensate water (settling tank)	<0.1	<0.1	0.5	2.1	3.4	6.0
	Effluent flowing into manhole	<0.1	0.3	0.5	0.5	1.8	3.1
5	CN Yard background (settling tank) (2-4 h composite)	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1
	Boiler blowdown (grab sample)	0.3	0.8	2.4	0.3	<0.1	3.8
	Storm sewer (2-4 h composite)	0.8	3.8	4.1	2.3	1.2	12.2
6	Effluent from ditch near storage tank (settling tank) (grab sample)	1.8	3.5	6.3	13.8	<0.1	25.4
	Boiler blowdown (grab sample)	0.3	0.8	3.6	11.4	<0.1	16.1
	Sludge from storage tank (grab sample)	119.0	289.0	214.0	432.0	140.0	1194.0

[a] Sample 6-8 h composite unless specified otherwise.

Table 2-14. PCDFs detected in 2,4,5-T and 2,4-D formulations.

Formulation	Production date	Location	Active Ingredient ($g.L^{-1}$)	Homologues ($mg.kg\text{-}formulation^{-1}$)	Total PCDF ($mg.kg\text{-}formulation^{-1}$)	Ratio of PCDF/PCDD[a]	Reference
2,4,5-T ester	1967	Europe	240		< 0.05		Rappe et al. (1978b)
"	1966	"	500		< 0.05	< 0.5	"
"	1967	"	165	T_4CDF (not 2,3,7,8-)	0.11	0.5	"
"	1967	"	750	T_4CDF (not 2,3,7,8-)	0.15	0.83	"
"	1962	"	865		< 0.05	< 0.05	"
Agent Orange (1:1 mix of 2,4-D and 2,4,5-T butyl esters): 3 samples analyzed	?	USA	450-500		< 0.05	< 0.17	"
	?	"	"	T_3CDF: 0.10 T_4CDF (not 2,3,7,8): 0.40 P_5CDF:0.2	0.70	0.39	
	?	"	"		< 0.05	< 0.01	"
2,4,5-T acid	1952	Sweden	?	T_4CDF: 0.06 P_5CDF: 0.01	0.07	0.15	Norström et al. (1979a)
"	?	"	?		< 0.05	< 0.1	"
"	?	"	99%		< 0.05		"
"	1960	"	?		< 0.05	0.12	"
"	?	USA	?		< 0.05	< 0.01	"
2,4,5-T acetate	<1977	?	500	T_4CDF: 0.13 P_5CDF: 0.008	0.138	4.6	Ahling et al. (1977)
2,4-D ester	<1964	Sweden	?		< 0.05		Norström et al. (1979a)
"	<1960	"	?		< 0.05		"
2,4-D	1964	"	?	0.06 T_4CDF	0.06	> 1.2	"
2,4-D	1964	"	?		< 0.05		"

[a] Where no number is given, TCDD was not detected and the detection limit was not specified.

contamination; the ratio of PCDFs:PCDDs in a given formulation ranges from <0.01 to 4.6.

Concern has been expressed about the possible formation of dioxins and furans from the burning of phenoxy herbicides. Ahling et al. (1977) burned 2-butoxyethyl 2,4,5-trichlorophenoxyacetate at 500°C to 850°C (transit times of ~0.7 seconds, wood chips used as fuel) with oxygen, in an oxygen deficient atmosphere and under simulated open fire conditions (formulation mixed with wood chips and leaves and allowed to burn at ~100°C). In all except the "open fire" experiment, 99.995% of the parent compound was destroyed (Table 2-15). The control levels of PCDFs were surprisingly high; up to 0.5 mg was emitted during an experiment. It is possible, for example, that PCDFs could be formed from, or be present in, CPs in the wood chips. Even so, the emissions of PCDFs were higher (up to 30 times) than the emissions of PCDDs. The authors could find no correlation between PCDF emission and either temperature or transit time. The emitted levels of PCDFs and PCDDs were apparently higher than those expected from typical levels in the original formulation (see Table 2-14). However the authors do not give mass balance data.

2.1.2.3 Other CP-Derived Herbicides

A variety of CP-derived phenoxy herbicides, other than 2,4-D and 2,4,5-T, have been produced and used in various countries. When these were analyzed, PCDDs or PCDFs were usually found.

For example, 1,3,5-trichloro-2-(4-nitrophenoxy) benzene (CNP), a herbicide widely used in Japan, has been reported to contain 178 to 202 mg-PCDD.kg-formulation^{-1} and 11 to 19 mg-PCDF.kg-formulation^{-1}. Homologues with 1 to 6 Cl atoms occurred, with T_4CDF dominant at 9.4 to 16 mg.kg-formulation^{-1} (Table 2-16) (Yamagishi et al. 1981). Freshwater fish from aquatic systems adjacent to areas sprayed with CNP were heavily contaminated (1.2 mg-CNP.kg-BW^{-1} and 0.2 µg-1,3,6,8-T_4CDD.kg-BW^{-1}).

Two other Japanese products, nitrofen [2,4-dichloro-1-(4-nitrophenoxy) benzene (NIP)] and chloromethoxynol [2,4-dichloro-1-(3-methoxy-4-nitrophenoxy) benzene (X-52)], contained from 0.07-1.3 mg-PCDD.kg-formulation^{-1} and 1.2-1.5 mg-PCDF.kg-formulation^{-1} (Table 2-16).

2.1.3 POLYCHLOROBENZENES

Polychlorobenzenes (PCBzs) are common environmental contaminants. They are used as pesticides and solvents and in the electrical and chemical industries (Choudry et al. 1983). Hexachlorobenzene (HCBz) has been used as a fungicide and is also encountered as a waste by-product from chlorinated hydrocarbon manufacturing plants. Villanueva et al. (1974) found three HCBz samples

Table 2-15. Formation of PCDFs on combustion of 2-butoxyethyl 2,4,5-T (after Ahling et al. 1977).

Temperature (°C)		500	675	850	500	625	850	750	100
% O_2		15	12	10.5	13	12	7.5	0	17.5
Emitted PCDF (mg.kg-formulation^{-1})[α]	T_4CDF	< 0.2	8.0	1.3	0.6	0.2	2.9	7.6	1.0
	P_5CDF	1.8	7.1	1.9	1.3	< 0.3	4.2	5.7	0.5
	H_6CDF	1.3	2.8	1.8	< 0.6	< 0.3	4.9	1.7	< 0.4
	H_7CDF	< 0.7	< 0.7	1.4	< 0.6	< 0.3	3.4	< 0.2	2.3
	O_8CDF	< 0.7	< 0.7	< 0.6	< 0.6	< 0.3	3.9	< 0.1	< 0.4
	Σ PCDF	> 3.1	> 17.9	> 6.4	< 1.9	> 0.2	> 19.3	> 15.0	> 3.8
Ratio PCDF/PCDD		0.6	7.8	30	1.5	0.5	12.1	4	1.5

[α] Control Σ PCDFs ranged up to 0.5 mg emitted during an experiment run time. The data are not corrected for these background levels.

Table 2-16. PCDFs in CP-derived herbicides other than 2,4-D and 2,4,5-T (data after Yamagishi et al. 1981).

Herbicide	Country of Use	PCDF Homologue
CNP[α]	Japan	Cl_1 ND
		Cl_2 0.19-0.35 mg.kg-formulation^{-1}
		Cl_3 0.41-0.73 "
		Cl_4 9.4-16 " (includes 2,3,7,8-)
		Cl_5 1.0-1.8 "
		Cl_6 0.2-0.28 "
NIP[α]	"	Cl_1 0.34-0.70 mg.kg-formulation^{-1}
		Cl_2 0.12-2.0 "
		Cl_3 0.47-0.96 "
		Cl_4 0.29-0.49 "
		Cl_5 ND
		Cl_6 ND
X-52[α]	"	Cl_1 0.48 mg.kg-formulation^{-1}
		Cl_2 0.21 "
		Cl_3 0.45 "
		Cl_4 0.32 "
		Cl_5 0.08 "
		Cl_6 ND

[α] See Section 2.1.2.3.

to be contaminated with 0.35 to 58.3 mg-$O_8CDF.kg^{-1}$; H_7CDF was also detectable in one sample (>0.2 $mg.kg^{-1}$).

Buser (1979) showed that tetra through octa PCDF congeners were formed when tri-, tetra- or pentachlorobenzenes were pyrolyzed at 620°C in air. The levels produced are summarized in Table 2-17. The lower the degree of chlorination of the chlorobenzene, the more complete was its degradation; T_3CBz was >95% degraded, T_4CBz >90% and P_5CBz ≈50%. Mass fragmentograms of the combined pyrolyzate showed the elution of a complex isomer mixture, which suggested the formation of PCDFs by several routes. The more toxic PCDF isomers (2,3,7,8-, 1,2,3,7,8- and 2,3,4,7,8-) were present but not as major components. Since the formation reactions are bimolecular, they would be expected to be dependent on concentration; i.e. relatively less PCDF would be formed at the lower chlorobenzene concentrations likely to occur in incinerators.

Chlorobenzenes have been observed in municipal incinerator emissions (Section 2.2.1). Buser (1979) stated the amount of PCBzs in municipal incinerators may be too low to be of primary concern for PCDF or PCDD formation. There would be a higher chance of PCDDs or PCDFs being produced if chlorobenzene industrial wastes or residues are incinerated. It is conceivable that incineration of PVC would yield PCBzs and hence PCDFs and PCDDs.

Choudry et al. (1983) speculated that water bodies contaminated with PCBzs would also be likely to contain phenols. They therefore studied the photoreactions of PCBzs in aqueous acetonitrile containing phenols, in the laboratory at >285 nm. PCDFs with 2 Cl less than the starting PCBs were produced at yields up to about 1%. It is not known whether such reactions could occur in the environment.

2.2 INCINERATION AND COMBUSTION

2.2.1 MUNICIPAL INCINERATORS

Mechanistic aspects of the thermal formation of halogenated organic compounds from chlorinated organic precursors, including the PCDFs, have been reviewed by Choudry and Hutzinger (1982).

The process of incineration, as related to PCDDs and similar compounds, has been reviewed by Lustenhower et al. (1980). Incineration is a generally accepted method for disposal of municipal waste. Raw refuse is burned at 800 to 1100°C so that the bulk is reduced to slag and fly ash while the flue gases are released to the environment. Modern incinerators usually contain electrostatic precipitators that retain more than 98% of the particulate matter, which might otherwise be released with the flue gases (Lustenhower et al. 1980). The quench water used to cool the slag is also a possible source of environmental contamination. It is generally accepted that much organic material is not completely combusted at the temperatures common in municipal incinerators and, on the other hand, that new compounds are generated

Table 2-17. PCDFs produced by pyrolysis of PCBzs (after Buser 1979).

Chlorobenzene	Ratio and total sample weight	PCDF ng.sample^{-1}				
		T_4	P_5	H_6	H_7	O_8
1,2,3-T_3CBz	1:1:1					
1,2,4-	200 µg	400	1100	550	50	< 5
1,3,5-						
1,2,3,4-T_4CBz	1:1:1					
1,2,3,5-	200 µg	< 2	5	160	450	200
1,2,4,5-						
P_5CBz	200 µg	< 2	< 5	< 5	5	30
Combined "above"	1:1:1:1:1:1:1 200 µg	80	600	1100	600	60

from the garbage being incinerated. Such compounds could include chlorinated organics formed from carbonaceous fuels and endogenous chlorine.

PCDFs and PCDDs were first reported to be present in the fly ash of municipal incinerators in the Netherlands by Olie et al. (1977). Subsequently, PCDDs have been reported in incinerator effluents from many countries (NRCC 1981a). PCDFs have also been extensively reported (Table 2-18). There are fewer international data on the occurrence of PCDFs in particulate and flue gas than in fly ash. Both PCDDs and PCDFs have been found in most of the incinerator residues examined and PCDFs often occur at higher levels (e.g. Buser et al. 1978a; NRCC 1981a; Liberti et al. 1982a).

All the higher PCDF homologues (tetra through octa) occur in combinations in incinerator effluents (Table 2-18), although the relative proportions of each homologue can differ with the location. The more toxic PCDF isomers (2,3,7,8-, 1,2,3,7,8- and 2,3,4,7,8-PCDF) tend to be major constituents (Table 2-18; Buser et al. 1978a). This is in contrast to the PCDDs, where the more toxic isomers are present only as minor constituents (NRCC 1981a).

The range of reported concentrations for each homologue and for total PCDF varies considerably between locations and between sampling times at the same location. In some cases this variation may in part be due to differences in analytical technique or standards. Lustenhower et al. (1980) reported PCDF residues at one location at different times. The variation in total concentration was small and the homologue ratios were similar at each measurement (Table 2-19). The type of incineration and the operating conditions were not specified. However, when Rappe et al. (1983a) analyzed four samples taken at two sampling times from a Swedish fluidized-bed incinerator, there was a considerable variation in both total concentration and homologue ratios between the sampling periods (Table 2-20). They reported that neither PCDDs or PCDFs were the dominant polynuclear aromatics formed. Of the lower Cl homologues, PCDFs predominated over PCDDs while this was reversed for the octa homologues (Table 2-21). Rappe et al. (1982) also noted higher PCDF levels in the condensate than in the particulate, but speculated that the samples may have been taken before equilibration (Rappe 1983). Gizzi et al. (1982) reported a 10-fold variation in the amount of PCDF (and PCDD) emitted from an Italian urban incinerator over a period of 9 months; in 17 samples collected and analyzed in the same way, PCDF emissions from the stack ranged from 3.9 $mg.h^{-1}$ to 324 $mg.h^{-1}$.

Data on PCDF levels associated with Canadian incinerators are limited. It seems reasonable to assume that where PCDDs have been detected in Canada, PCDFs will occur in concentrations at least as high. PCDDs have been detected at several Canadian locations (e.g. Karasek 1980; Eiceman et al. 1981; Karasek et al. 1982). Concentrations are generally at least as high as those reported internationally.

Table 2-18. Some examples of PCDFs detected in international municipal incinerator emissions.

Type of Incinerator	Location	Operating Conditions	Sample Type	Sample Size	Homologues (isomers)	Concentration $\mu g.kg^{-1}$	Total PCDF $\mu g.kg^{-1}$	Reference
Municipal	Switzerland		Stack-fly ash		T_4CDF	1	85	Buser et al. (1978a)
					P_5CDF	4		
					H_6CDF	30		
					H_7CDF	40		
					O_8CDF	10		
	"		Stack-particulates		T_4CDF	100	320	"
					P_5CDF	100		
					H_6CDF	70		
					H_7CDF	50		
					O_8CDF	ND[a]		
Municipal	Switzerland		Flue gas stream (260° C flue temperature)		T_4CDF (major 2,3,7,8-)		100	Buser et al. (1978b)
					P_5CDF (including 1,2,3,7,8-; 2,3,4,7,8-; 2,3,4,6,8-; 1,2,3,4,8- and possibly 1,3,4,7,8-)			
					H_6CDF (including 2,3,4,7,8-)			
Fluidized bed municipal	Sweden	700°C, ≈ 2 s retention	Particulate	3 m^3	see Table 2-20		191-389	Rappe et al. (1983a)
					2,3,7,8-T_4CDF	17-35		
"	"	"	Condensate	"	see Table 2-20		178-2136	"
					2,3,7,8-T_4CDF	4-9		
Municipal	Netherlands		Electrostatic precipitate (range from 3 analyses of 2 installations)		T_4CDF	169-315	1407-2299	Kooke et al. (1981)
					P_5CDF	364-560		
					H_6CDF	555-870		
					H_7CDF	287-495		
					O_8CDF	32-89		
Various			Electrostatic precipitate (range from 80 analyses of 25 installations)		T_4CDF	15-223	278-1976	Lustenhower et al. (1980)
					P_5CDF	45-510		
					H_6CDF	109-810		
					H_7CDF	89-407		
					O_8CDF	20-26		
			Stack particulate matter		T_4CDF	460	4290	"
					P_5CDF	960		
					H_6CDF	1600		
					H_7CDF	1130		
					O_8CDF	140		

Table 2-18. (CONT'D)

Type of Incinerator	Location	Operating Conditions	Sample Type	Sample Size	Homologues (isomers)	Concentration $\mu g.kg^{-1}$	Total PCDF $\mu g.kg^{-1}$	Reference
Municipal	Milan		Fly ash		T_3CDF	ND	142.8	Liberti et al. (1982a)
					T_4CDF	10.9		
					P_5CDF	29.4		
					H_6CDF	37		
					H_7CDF	50.5		
					O_8CDF	15		
"	Florence		Fly ash		T_3CDF	60	2610	"
					T_4CDF	285		
					P_5CDF	415		
					H_6CDF	910		
					H_7CDF	715		
					O_8CDF	125		
"	Rome		Fly ash		T_3CDF	ND	863	"
					T_4CDF	576		
					P_5CDF	216		
					H_6CDF	57		
					H_7CDF	13		
					O_8CDF	1.5		
"	Rome		Fumes		T_3CDF	1218	2736	"
					T_4CDF	994		
					P_5CDF	373		
					H_6CDF	135		
					H_7CDF	16		
					O_8CDF	ND		
"	Messina		Sludge		T_3CDF	ND	735	"
					T_4CDF	247		
					P_5CDF	358		
					H_6CDF	93		
					H_7CDF	34		
					O_8CDF	3		
Municipal	Netherlands		Electrostatic precipator fly ash		T_4CDF	314	2044	Helder et al. (1982)
					P_5CDF	500		
					H_6CDF	660		
					H_7CDF	484		
					O_8CDF	86		
Muncipal	Italy		Fly ash (range at 6)		T_4CDF	< 0.01-61		Cavallaro et al. (1982)
					O_8CDF	< 0.01-255		
Rotary Kiln municipal	West Germany	Average after burner temperature = 1040°C	Fly ash (electrostatic precipitate) (3 samples over 3 months)		T_4CDF	0.8-28	2.6-68	Karasek and Viau (1983)
					P_5CDF	0.3-8		
					H_6CDF	0.4-8		
					H_7CDF	0.6-10		
					O_8CDF	0.3-10		

[a] ND = Not detected.

Table 2-19. Variation of PCDF concentrations in fly ash from one incinerator[α] during a 4-week period (Lustenhower et al. 1980).

Homologue	PCDF Content of fly ash (μg.kg^{-1}) Week 1	2	3	4	%Contribution Mean ± S.D.
T_4CDF	147	50	111	68	10.7 ± 4.2
P_5CDF	239	153	196	180	22.2 ± 2.4
H_6CDF	345	282	361	357	39.1 ± 2.3
H_7CDF	202	202	177	291	25.4 ± 0.7
O_8CDF	18	21	18	31	2.6 ± 0.7
Total	951	708	863	927	

[α] Type and operating conditions not specified.

Table 2-20. Variation of PCDF concentrations between two sampling times at a municipal incinerator[α] (after Rappe et al. 1983a).

Homologue	Condensates 1 µg.kg^{-1}	Condensates 1 number isomers	Condensates 1 % contribution	Condensates 2 µg.kg^{-1}	Condensates 2 number isomers	Condensates 2 % contribution	Particulates 1 µg.kg^{-1}	Particulates 1 number isomers	Particulates 1 % contribution	Particulates 2 µg.kg^{-1}	Particulates 2 number isomers	Particulates 2 % contribution
$\sum T_4$CDF	205	20	73.7	1574	20	73.7	110		33.8	75	-	48.4
2,3,7,8-	17	-	6.1	35	-	1.6	9	-	2.8	4	-	2.6
$\sum P_5$CDF	17	4	6.1	260	4	12.2	30	5	9.2	13	2	8.4
$\sum H_6$CDF	30	3	10.8	240	6	11.2	75	5	23.1	13	3	8.4
$\sum H_7$CDF	22	3	7.9	45	3	2.1	74	3	22.8	25	3	16.1
O_8CDF	4	-	1.4	17	-	0.8	27	-	8.3	25	-	16.1
TOTAL	278			2136			325			155		

[α] Fluidized bed, operating at ≈ 700°C with 2 s retention time.

Table 2-21. Relative quantities of PCDFs and PCDDs in incinerator residues[α] (after Rappe et al. 1983a).

Homologue	Ratio Σ PCDF/Σ PCDD			
	Condensates		Particulates	
	1	2	1	2
T_4CDF	>500	25	>100	>100
P_5CDF	> 30	> 250	> 60	> 25
H_6CDF	>300	>1000	>500	>200
H_7CDF	1	2	1.2	0.8
O_8CDF	0.36	0.68	0.6	0.83

[α] Municipal, fluidized bed, operating at ≈ 700°C with 2 s retention time.

Wang et al. (1983) analyzed stack samples from a coal-fired thermal station, a central heating plant, a heat recovery incinerator and a controlled-air municipal incinerator. Preliminary data suggest that PCDDs and PCDFs are present in the fly ash of the municipal incinerator, the heat recovery incinerator and the coal-fired thermal station. Concentrations for the PCDF isomers were reported to be typically 2-5 times higher than for the corresponding PCDD isomer groupings. PCDD levels ranged from 30 to 900 $\mu g.kg^{-1}$.

Ozvacic et al. (1984) studied emissions of PCDDs and PCDFs from a municipal incinerator in Hamilton, Ontario. The unit burns pulverized solid municipal waste at temperatures as high as 1100 °C. Total PCDFs emitted with the stack gas were 6.2 ± 1.3 $\mu g.m^{-3}$ (or 101.6 $\mu g.s^{-1}$). Tetra through octa congeners were detected; the lower (tetra and penta) chlorinated congeners predominated. PCDF concentrations ranged from 22 to 105 $\mu g.kg^{-1}$ (average 47 $\mu g.kg^{-1}$) in the precipitator fly ash and from <0.1 to 3.9 $\mu g.kg^{-1}$ (average 0.6 $\mu g.kg^{-1}$) in the bottom ash. Penta congeners predominated in the precipitator ash, followed by tetra, hexa, hepta and octa in that order. The octa congener was not detected in the bottom ash samples. PCDDs were present at lower concentrations than the PCDFs. PCDFs were also detected in the refuse being fed to the incinerator at concentrations from <0.1 to 25 $\mu g.kg^{-1}$ (average 2.3 $\mu g.kg^{-1}$). PCDDs occurred at higher concentration in the refuse. No congener was clearly dominant in the refuse. Possible precursors detected in the refuse included PCBs, chlorobenzenes and chlorophenols.

Clement et al. (1984) analyzed PCDD and PCDF emissions from a municipal incinerator in Toronto, Ontario. The incinerator burns refuse at about 1010 °C. PCDFs were not detectable in the feedstock (<0.1 $\mu g.kg^{-1}$). Possible precursors detected included chlorobenzenes, chlorophenols and PCBs. Total PCDF levels in the precipitator fly ash ranged from 280 to 1800 $\mu g.kg^{-1}$ (average 1127 $\mu g.kg^{-1}$) over 3 tests. Hexa congeners predominated, followed by hepta, penta, tetra and octa in that order. Total PCDFs emitted with the stack gas averaged 0.84 $\mu g.m^{-3}$ with tetra and hexa congeners dominant.

Environment Canada (1983c) reported that ash samples from several coal-fired generating stations contained very low levels of PCDFs (<1 $\mu g.kg^{-1}$ for any isomer group; penta congeners were dominant) and generally no detectable PCDDs (<20 $ng.kg^{-1}$).

While the presence of dioxins and furans in incinerator effluents is accepted as ubiquitous, their sources are not clearly defined. One theory, the "trace chemistries of fire hypothesis", states that PCDDs and PCDFs are simply among the numerous products that are formed during combustion. This theory, developed by Dow Chemical Co. (1978), has been reviewed and evaluated in NRCC (1981a). However, it is conceivable that the PCDDs and PCDFs formed may have been associated with PCBs or hexachlorobenzene in the air. It is also surprising that O_8CDD was a major product if de novo chlorination was the route of formation; O_8CDD is a major product from the combustion of

hexachlorobenzene. The de novo formation of PCDFs may be more likely, as the lower chlorinated PCDFs often predominate in incinerator effluents (see Table 2-18).

It is also highly probable that sources of PCDD and PCDF are present in municipal garbage, because trace contaminants of herbicides, herbicide containers, treated wood, products containing PCB, etc. are all routinely disposed of in municipal garbage. As has been discussed (Section 2.1), the incineration process could actually increase the level of PCDF and PCDD contamination from such products. Karasek and Viau (1983) report a correlation between the levels of PCDFs and PCDDs and other chlorinated compounds (chlorobenzenes, PCBs, etc.) in a municipal incinerator effluent.

There is no clear evidence for any route of PCDF formation during municipal garbage incineration. Buser et al. (1978a, b) found similar isomers present at such different facilities as an industrial heating facility and a municipal incinerator. This suggests similar sources, thus lending some support to the "trace chemistries of fire" hypothesis. However they also found the isomer pattern in a municipal incinerator to be similar to the isomer pattern from the pyrolysis of commercial PCB samples. Lustenhower et al. (1980) reported no detectable levels of PCDFs in compost samples that they say represented all but the bulk components of refuse. However they point out the limitations of their experiment. For example, little is known about microbial degradation of PCDFs (see Chapter 4) and it is conceivable that PCDFs biodegraded in the compost or diluted to below detection limits.

Taylor et al. (1983) investigated the feasibility of the various proposed mechanisms of PCDD/PCDF formation during combustion processes. They sampled the municipal wastes being fed to an incinerator (700-750°C combustion) as well as various incinerator effluents. While recognizing it is "virtually impossible" to obtain a representative homogenous sample of the incinerator feed, they reported that most of the PCDDs and PCDFs present in the combustion products, were not present in the waste.

It is quite likely that the PCDFs in municipal incinerator residues arise from multiple sources.

2.2.2 INDUSTRIAL INCINERATORS AND ELECTRICAL POWER PLANTS

PCDFs have been found in fly ash and flue gases of industrial incinerators and heating facilities (e.g. Buser et al. 1978a). In situations where a specific chemical is being combusted, the data are reported in the section of Chapter 2 discussing that chemical.

No PCDDs or PCDFs were detected in the ashes from a variety of Canadian electrical power plants (EPS 1982). The detection limit was

stated as ~50 $\mu g.kg^{-1}$ but the extraction efficiency on actual ash samples is not known. The authors stated that there are not enough data to conclude whether or not power plant ashes are a source of PCDDs or PCDFs. An ash sample from an Ontario Hydro incinerator contained tetra through octa PCDF homologues in the concentrations indicated in Table 2-22. No PCDDs or PCDFs were detected in effluents from a coal/refuse burning facility operating at 1200°C for large scale conversion of heat to electricity.

The case where high levels of PCDFs were associated with a wire-reclamation incinerator (Hryhorczuk et al. 1981) has been discussed (Section 2.1.1.1).

2.3 ESTIMATED RELATIVE IMPORTANCE OF THE SOURCES OF PCDF INPUT TO THE ENVIRONMENT

Where possible, the data used in the following estimates are pertinent to Canada. However, in some cases, few Canadian data are available. It is impossible to calculate absolutely the potential quantities of PCDF input to the environment. The following data are intended only to indicate the magnitude of the problem.

2.3.1 PCB-DERIVED PCDFs

Approximately 6.5×10^8 kg of Aroclor were produced and distributed in North America between 1930 and 1975 (Brinkman and Dekok 1980). Additionally, PCBs were imported from France and other countries. If the PCBs are assumed to contain an average 5 mg-PCDF.kg^{-1}, with 20-50% of this the 2,3,7,8-isomer (Table 2-1), then at least an estimated 3.2×10^3 kg of PCDF, including up to 1.6×10^3 kg of 2,3,7,8-T_4CDF, are in use or have been input to the North American environment. Obviously, Canadian sales were lower than U.S. sales. The PCBs originally used in Canada, prior to controls, have been estimated at 3.5×10^7 kg (Environment Canada 1983b), much of which is now distributed in the environment. This could account for 175 kg of PCDF, using the assumptions described above. Environment Canada (1983a) identified over 1.7×10^7 kg of PCB in the Canadian national inventory as of 1982. This could represent 85 kg of PCDF, including perhaps 42.5 kg of 2,3,7,8-T_4CDF, using the same assumptions. These figures for PCDFs may be an underestimate. When PCBs are subjected to heat, their PCDF content rises. Pyrolyzed PCBs contain even higher concentrations of PCDFs (Table 2-3). The percentage of PCBs used in equipment that could subject them to elevated temperatures is high; transformer and condenser applications accounted for more than 70% of Canadian PCB sales. In 1982, electrical applications accounted for 99.9% of the Canadian PCB inventory (Environment Canada 1983a).

Table 2-22. Concentration of PCDFs in Ontario Hydro incinerator ash sample (EPS 1982).

Homologue	Concentration ($\mu g.kg^{-1}$)
T_4CDF	0.33
P_5CDF	1.12
H_6CDF	0.35
H_7CDF	0.14
O_8CDF	0.11
Total	2.05

2.3.2 CP-DERIVED PCDFs

In the order of 2900 tonnes of P_5CP are used in Canada each year (NRCC 1981a). If P_5CP is assumed to contain 280 mg-PCDF.kg^{-1} (Table 2-8), then 800 kg of total PCDF could enter the environment each year. This figure may be an overestimate since much of the PCDF-treated wood is exported. However, the incineration of treated wood or wood products could result in elevated concentrations. The occurrence of 2,3,7,8-T_4CDF has not been documented in P_5CP, but some of the other relatively toxic isomers are known to occur (Table 2-9).

There is a very limited data base for PCDF concentrations in the CP-derived phenoxy herbicides 2,4-D and 2,4,5-T. There are no data on PCDF levels in herbicide formulations in current use. The relative importance of the phenoxy herbicides, as a source of PCDFs for the Canadian environment, is probably low.

PCDFs occur in relatively high concentrations in 2,4,6-T_3CP (up to 100 mg.kg^{-1}), which suggests that manufacturing plants using T_3CP could be a contamination source. This would include phenoxy herbicide manufacture. The highly toxic 2,3,7,8-T_4CDF congener is known to be a major contaminant of 2,4,6-T_3CP.

2.3.3 COMBUSTION-DERIVED PCDFs

An estimated annual total 81 x 10^3 tonnes of precipitated and 7637 tonnes of emitted matter are produced in Canada (NRCC 1981a). These estimates were based on 1976 data on the amount of refuse burnt and on average estimated emissions for the type of facility and are necessarily rough. The other documented incineration processes (sewage, industrial, wood burning, etc.), which are potentially significant sources of PCDF, have not been included in the following calculations.

Using the "average" figure of ~ 1000 µg-PCDF.kg^{-1} (based on Canadian and International data), 88 kg of total PCDF are potentially input to the environment. The data indicate that 2,3,7,8-T_4CDF may occur in significant amounts.

2.4 PCDF RESIDUES IN THE ENVIRONMENT

The potential impact of the PCDF sources described in Sections 2.1 and 2.2 cannot be assessed unless the PCDF pollution levels in environmental matrices, food sources and, perhaps ultimately, human tissue are determined. As discussed in NRCC (1981a), it is only in recent years that there has been any concerted effort to identify the presence and levels of the PCDDs. The available data for the PCDFs are even less informative.

2.4.1 AQUATIC SYSTEMS

There is little isomer-specific information available on PCDF residues in aquatic matrices, including edible organisms. Data that are not isomer-specific are of little use from a toxicological viewpoint. Canadian data are particularly sparse and extrapolation must often be made from U.S. data. The useful data that are available are summarized below.

Stalling et al. (1983) reported PCDF residues in several types of fish from various locations in the Great Lakes and rivers in their watersheds. They reported 12 to 153 $ng.kg^{-1}$ of total PCDF in various types of fish sampled (carp, trout, walleye, bloater) at various locations in Lakes Huron, St. Clair, Erie, Superior and Ontario. Homologues included tetra through octa PCDFs, but not all of the analyses were isomer-specific.

Stalling (1980) and Smith and Stalling (1981) report that fish from various U.S. locations had from 36 to 1700 $ng.kg^{-1}$ total PCDFs, including 2,3,7,8- and other highly toxic isomers (Table 2-23).

Preliminary data (Ryan et al. 1983), which was not confirmed, showed significant quantities of PCDFs (up to 188 $ng.kg^{-1}$) tentatively including the toxic 2,3,7,8-isomer (13 to 79 $ng.kg^{-1}$) in fish from Canadian Great Lakes locations (Table 2-23).

Stalling et al. (1982) reported PCDF residues in fat from snapping turtles from the Hudson River and fat from seals from the Baltic Sea (Gulf of Bothnia). The turtle fat contained a total of 3 $\mu g\text{-PCDF}.kg^{-1}$ (including 45 $ng.kg^{-1}$ 2,3,7,8-; 620 $ng.kg^{-1}$ 2,3,4,7,8- + other penta through octa isomers) and the seal fat, 40 $ng\text{-PCDF}.kg^{-1}$ (including 1 $ng.kg^{-1}$ 2,3,7,8-; 15 $ng.kg^{-1}$ 2,3,4,7,8- + other penta through octa isomers).

Petty et al. (1983) and Stalling et al. (1983) investigated PCDD and PCDF residues in sediments and fish of the Hudson and Houstatonic rivers (Woods Pond, MA). While the hepta and octa congeners predominated in sediment samples, the tetra and penta congeners were dominant in fish samples (Table 2-24). A comparison of these data with those for the PCDF isomers present in Aroclor 1260, a suspected source of the PCDFs in the river, revealed the Aroclor 1260 congener patterns were not parallel with those in either sediment or fish. Stalling et al. (1983) speculated that the observed differences in PCDF residues in sediment and fish could result from the lower water solubility of the hepta and octa congeners relative to the tetra and penta congeners or from large differences in adsorption on sediments. More data are needed to understand (and hence perhaps to predict) the residue profiles in the environment.

Lake et al. (1981) reported the presence of 2,3,8-T_3CDF in Naragansett Bay, R.I. Suspended particulates from the upper bay

Table 2-23. Summary of isomer-specific data on PCDFs in organisms from North American waters.

Location	Sample	Homologue	ng.kg^{-1}	Total ng.kg^{-1}	Reference
Hudson	Yellow perch (whole fish composite)	2,3,7,8-	1000	1723	Stalling (1980)
		Other tetra-	150		
		1,2,3,7,8-	100		
		2,3,4,7,8-	170		
		Other penta-	108		
		1,2,3,6,7,8-	170		
		Other hexa-	22		
		Hepta-	3		
		Octa-	< 5		
Woods Pond, MA	Striped bass (whole fish composite)	2,3,7,8-	18	50	"
		Other tetra-	1		
		1,2,3,7,8-	4		
		2,3,4,7,8-	12		
		Other penta-	-		
		1,2,3,6,7,8-	12		
		Hepta-	< 2		
		Octa-	< 3		
Edenton	Striped bass (whole fish composite)	2,3,7,8-	21	44	"
		Other tetra-	1		
		1,2,3,7,8-	3		
		2,3,4,7,8-	4		
		Other penta-	> 2		
		1,2,3,6,7,8-	4		
		Other hexa-	3		
		1,2,3,4,6,8,9-	6		
		Octa-	< 3		
Chesapeake Bay	Striped bass (whole fish composite)	2,3,7,8-	13	36	"
		Other tetra-	1		
		1,2,3,7,8-	2		
		2,3,4,7,8-	7		
		Other penta-	> 2		
		1,2,3,6,7,8-	7		
		Other hexa-	-		
		Hepta-	4		
		Octa-	< 3		
Cooper River	Striped bass (whole fish composite)	2,3,7,8-	20	64	"
		Other tetra-	24		
		1,2,3,7,8-	5		
		2,3,4,7,8-	5		
		Other penta-	-		
		1,2,3,6,7,8-	5		
		Other hexa-	-		
		Hepta-	4		
		Octa-	< 3		

Table 2-23. (CONT'D)

Location	Sample	Homologue	ng.kg^{-1}	Total ng.kg^{-1}	Reference
Mouth Cayaga Creek, Niagara River	Carp (whole fish composite)	2,3,7,8-	5	1119	Smith and Stalling (1981)
		Other tetra-	5		
		2,3,4,7,8- or 1,3,4,7,9-	80		
		1,2,3,4,7,8-	203		
		Other hexa-	22		
		1,2,3,4,6,7,8-	306		
		1,2,3,4,6,8,9-	22		
		Octa-	388		
Fort Niagara, Niagara River	Carp (whole fish composite)	2,3,7,8-	6	278	"
		2,3,4,7,8- or 1,3,4,7,9-	66		
		1,2,3,7,8-	4		
		1,2,3,4,7,8-	135		
		1,2,3,4,6,7,8-	44		
		1,2,3,4,7,8,9-	7		
		Octa-	6		
Lake Ontario (mainly Kingston, Bay of Quinte, Thousand Islands area) (1979-1980)	Smelt (4 samples fillet)	2,3,7,8-	3-34		Ryan et al. (1983)
		Other tetra-(2)	112[α]		"
	Catfish (fillet)	2,3,7,8-	54		"
		Other tetra-(1)	45		"
	White Perch (fillet)	2,3,7,8-	15		"
	Lake trout (fillet; 2 samples)	2,3,7,8-	8-24		"
	Rainbow trout (fillet)	2,3,7,8-	12		"
		Other tetra-(2)	188		"
	Ont. Salmon (fillet)	2,3,7,8-	79		"
		Other tetra-(1)	74		"
Saginaw Bay, Lake Huron	Carp[β] (whole fish composite)	2,3,7,8-	11	24-40	Stalling et al. (1983)
		2,3,6,7-	2-3		
		Other tetra-	< 1		
		1,2,4,7,8-/ 1,3,4,7,8-	2-3		
		1,2,3,7,8-	1-5		
		2,3,4,7,8-	4-11		
		1,2,3,4,7,8-/ 1,2,3,6,7,8-	2-5		
		1,2,3,4,6,7,8-	3-4		
		Octa-	4-8		

[α] In one sample only.

[β] 2 composite samples.

Table 2-24. Residues of PCDF homologues in samples from Woods Pond, MA. (data after Petty et al. 1983).

Homologue	ng.kg^{-1}[α]		
	Sediment	Yellow perch	Aroclor 1260
T_4CDF	<0.005 (<0.37)[α]	1.06 (49.3)	290 (6.84)
P_5CDF	0.009 (0.67)	0.64 (29.7)	1330 (31.38)
H_6CDF	0.15 (11.11)	0.44 (20.5)	1810 (42.69)
H_7CDF	0.92 (68.15)	<0.005 (<0.23)	780 (18.40)
O_8CDF	0.27 (20)	<0.005 (<0.23)	29 (0.68)
Total	1.35	2.14	4240

[α] Homologue as percent of total expressed in brackets.

contained 0.25 ng.kg^{-1}, mussels contained 52-1180 µg.kg^{-1} (decreasing with the distance of the sampling site from the top of the bay), clams from the mid-bay contained 26 µg.kg^{-1} and lobster hepatopancreas from the lower bay contained 1260 µg.kg^{-1}. Lake et al. (1981) speculate that the source was a chemical plant on the Patuxet River (which drains into Narragansett Bay) where a T_3CDF had previously been detected in the plant's wastewater.

Tissues from herring gulls from Saginaw Bay, Lake Huron were contaminated with about 26 ng-PCDF.kg^{-1}, including the more toxic isomers (Table 2-25) (Stalling et al. 1983).

2.4.2 OTHER MATRICES

Little information is available for other than aquatic matrices. The occurrence of PCDFs in food is discussed in the following section.

McLaughlin and Pearson (1984) measured concentrations of PCDDs and PCDFs in soil sampled 70 to 2480 m, in several directions, from the SWARU municipal incinerator in Hamilton, Ontario (see Section 2.2.1). Six of eleven samples were positive for PCDFs (0.01 to 0.18 µg.kg^{-1}). The two urban "control" samples were also positive for PCDFs (0.02 and 0.15 µg.kg^{-1}), but the rural "control" sample was negative. Tetra, hepta and octa homologues predominated. All of the samples, including the three "controls", were positive for PCDDs (0.05 to 4.64 µg.kg^{-1}). Only one site had detectable T_4CDD (0.007 µg.kg^{-1}). The samples showed no concentration gradient or deposition pattern consistent with either prevailing winds or the location of the calculated maximum ground level concentration. The authors concluded that emissions from the incinerator (operating since 1973) have not accumulated in adjacent surface soils.

There are some data on PCDD and PCDF levels in Ontario "raw" drinking water samples (OME 1984). Detailed information should be released soon.

2.4.3 FOOD FOR HUMAN CONSUMPTION

Inadequate data are available on the levels of PCDFs, or of PCDDs, in food from Canadian or other sources. Given that PCDFs may be ubiquitous in the industrialized environment, it is very likely that they occur in certain foods. PCDFs, including the more toxic isomers, are known to occur in aquatic systems and to accumulate in aquatic organisms so that fish and shellfish might be a significant dietary source. As was the case with the Yusho incidents (see Chapter 7), accidental spillage of contaminated fluids or the use of contaminated containers for shipping of human or animal feed could result in direct contamination of human food or indirect contamination via animal feed.

Table 2-25. PCDF residues in herring gull tissue[α] from Saginaw Bay, Lake Huron (after Stalling *et al.* 1983).

Isomer	ng.kg^{-1}
2,3,7,8-	2 - 4
1,2,4,7,8-/1,3,4,7,8-	2
1,2,3,7,8-	2
2,3,4,7,8-	16 - 10
1,2,3,4,7,8-/1,2,3,6,7,8-	7
1,2,3,4,6,7,8-/1,2,3,4,6,8,9-	4
Octa	8
Total	26 - 27

[α] Tissue (unspecified) samples from two gulls.

The use of treated wood products or by-products in agriculture or gardens, the use of PCDF contaminated herbicides and the use of treated wood waste in the pulp industry (leading to the possible use of contaminated containers) are other potential sources.

PCDDs have been demonstrated to occur in food, e.g. in some samples of beef fat in the U.S., fish in Lake Ontario, chicken livers in Canada and commercial gelatin in the U.S. (NRCC 1981a).

So far PCDF residues have been documented to occur in some similar matrices, including fish and shellfish (see Section 2.4.1).

Firestone (1977) reported levels of up to 0.4 $\mu g.kg^{-1}$ of each of H_6CDF and O_8CDF in 10 of 15 commercial gelatin samples assayed. The authors speculate the source to be from contamination of the animal hides used to make the gelatin with PCP.

Ryan and Pilon (1982) reported the presence of O_8CDFs in chicken livers from flocks raised on wood shavings contaminated with PCP and PCDDs. The O_8CDFs were detected in 6 of 8 samples tested, at levels from 0.04 to 0.13 $\mu g.kg^{-1}$.

3.0 RESIDUE METHODOLOGY

3.1 INTRODUCTION

The analytical methodology for determining residues of the 135 PCDF congeners is intimately associated with that for the 75 PCDD congeners. The two classes of compounds are neutral, planar, aromatic molecules, closely related in their chemical and physical properties. Consequently, the residues are extracted together by most of the analytical methods.

This critique closely follows the format and freely uses the information in NRCC (1981b), where published methodology for PCDDs was critically reviewed for the period 1970-80 and rated as to their degree of validation. Some of the papers included reference to PCDFs but most, if not all, of these PCDD methods will extract and include native PCDF residues in the final isolate.

Recent emphasis (since 1980) in method development has been on the separation of isomers by high-resolution gas chromatography (HRGC) with capillary columns, on mass spectrometry techniques such as NCI, and on the synthesis of the various PCDF isomers for use as standards. The elements of the methodology such as extraction or cleanup have not recently received much attention. Considering that pattern of method development, this critique will discuss first the separation and determination aspects of the methodology, and then the extraction and clean-up procedures for the various matrices. Data on published method summaries with ratings and on PCDF synthesis are provided in the appendices (I, II III, IV and V).

3.2 SEPARATION, DETECTION AND QUANTIFICATION

The source of the sample, degree of cleanup, residue (contaminant) level and presence of interferences such as PCB residues, are interrelated factors that must be considered when selecting the separation, detection and quantitation procedures. If isomer specificity at low $ng.kg^{-1}$ residue levels is desired, an extensive cleanup (e.g. Stalling et al. (1983) for fish oils) provides a final isolate suitable for high-resolution gas chromatography (e.g. 30 m DB-5 capillary column). At high $\mu g.kg^{-1}$ levels, for screening purposes, a simple cleanup (e.g. Buser (1975) for chlorophenol formulations) provides a suitable isolate for low-resolution gas chromatography (LRGC) (e.g. 2 m 3% OV-1 packed column). With such methods, specific congeners cannot be identified or homologues confirmed.

Table 3-1 lists most of the dimensions and operating conditions of GC columns developed for PCDF analysis. Many authors have not reported column properties such as retention index, theoretical plate number or height equivalent to a theoretical plate. This is unfortunate as it is difficult to fully evaluate the relative merits of

Table 3-1. Specifications[a] of some packed and capillary GC columns reported as used for PCDF analysis (for details, see Appendices II, III and IV).

Reference	Packed	Capillary
Vos et al. (1970)	1.8 m, 10% DC 200; 200°C	
Firestone et al. (1972)	2 m, 5% OV-101; 210-250° 2.2 m, 3% XE-60; 215°	-
Buser (1975)	2 m, 3% OV-1; 140-260°	-
Bowes et al. (1975b)	2 m, 3% OV-1, 5% SP-2401, 1.5% OV-17/1.95% QF-1, 3% OV-1/5% OV-210/6% OV-225; 195°, 200°, 192°, 213°	-
Nagayama et al. (1975)	2 m, 2% SE-30; 200°	-
Buser (1976b)	-	22 m, Silar 10C, OV-17, OV-101; 205°, 225°
Gray et al. (1975)	2 m, Dexsil 300, 1% Apolar 10C; 250°, 200°	
Miyata et al. (1977)	2 m, OV-17; 240-280°	-
Firestone (1977)	1 m, 3% OV-101, Silar 10C; 170-240°, 200°	-
Rappe et al. (1977)	-	22 m, OV-17; OV-101; 207°, 197°
Kuroki and Masuda (1978)	2 m, 3% Apeizon L, 5 m, 1.5% Apeizon L, 1.5% OV-17, 4% SF-96; 230°	-
Rappe et al. (1978c)	-	50 m, OV-17; 200-240°
Buser et al. (1978a)	-	50 m, OV-17; 100-250°, 120-250°
Gara et al. (1979)	1.5 m, OV-17; 250°	25 m, OV-17; 240°
Nostrom et al. (1979a)	OV-10; 120-300°	50 m, Silar 10C; 100-240°
Cavallaro et al. (1980b)	-	OV-17/OV-1, OV-101, 225°, 140-240°
Farrell (1980)	-	50 m, OV-101; 240°
Stalling et al. (1980)	-	50 m, OV-17, Silar 10C; 180-291°
Ryan and Pilon (1980)	-	15 m, SP-2100; 200°, 220°, 250°
Smith (1980)	2 m, OV-17; 180-285°, 180-270°	-
Kooke et al. (1981)	1.5 m, carbowax 20M; 170-250°	-
Rappe et al. (1981)	-	50 m, OV-17, Silar 10C; 210-250°
Liberti et al. (1982b)	2 m, 1.5% SP-2250/1.95% SP-2401; 200-245°	25 m, OV-1; 25-270°
Gizzi et al. (1982)	3% OV-1; 280°, 300°	-
Stanley et al. (1982)	-	carbowax 20M, SE-54; 60-300°
Mazer et al. (1983)	-	60 m, SP-2330, 30 m, SE-54; 200-250°, 200-275°
Korfmacher and Mitchum (1981)	-	50 m, SP-2100
Bell (1983)	-	60 m, SP-2330, DP-1; 240°
Rappe et al. (1982)	-	55 m, Silar 10C, 60 m, SP-2330; 25 m, SE-54
Ryan and Pilon (1982)	-	15 m, DB-5; 180-250°
Smith et al. (1982)		50 m, OV-275
Sakuma et al. (1983)		15m, DB-5; 80-160°, 160-280°

[a] Column specifications: length (m), liquid phase; isothermal or temperature program range (°C) (liquid phases in sequence if more than one).

one column over another and their performance between laboratories. Where data are provided as to a column's operating characteristics, they are usually based on PCDD standards (Table 2-8 in NRCC (1981b)).

Bowes et al. (1975b) reported the relative retention times of fifteen authenticated PCDF congeners for four packed columns. These data are summarized in Table 3-2 and provide an indication of the separation achievable for some PCDF congeners of the T_3, T_4 and P_5CDF series. Characteristically for packed columns, the homologues are distinctly separated; but as the data indicate, this is not so for the isomers. One could expect to find overlap of isomers in a homologue series when attempting to separate their residues in a complex mixture of sample concentrate by packed column GC.

Buser (1976b) evaluated three capillary columns of different polarities, OV-101, OV-17 and Silar 10C for PCDFs and PCDDs. The relative retention times of unspecified congeners (except octa standards) were determined (Table 3-3). These data indicate that the non-polar (OV-101) and medium-polar (OV-17) liquid phases separated the homologues. Some overlapping occurred with the polar Silar 10C liquid phase, but the resolution of isomers was better.

Mazer et al. (1983) recently synthesized the 38 tetra isomers and reported the relative retention times for separations on SP-2330 and SE-54 capillary columns. The data, summarized in Table 3-4, indicate that overlap between isomers occurs frequently.

Rappe and Buser (1980) reviewed, in part, their application of HRGC to the separation of PCDDs, but included a limited number of PCDFs. Unfortunately, there were insufficient standards available to establish the overall efficiency. Rappe et al. (1983b) noted that it was necessary to use a SP-2330 column to differentiate 2,3,7,8-T_4CDF from 2,3,4,8-T_4CDF as a Silar 10C column did not differentiate the isomers.

Progress in assessing the efficiency of separation of PCDF isomers by GC packed or capillary columns has been hindered by a lack of isomeric reference standards. Table 3-5 compares the number of congeners isolated from some sample matrices to the number of theoretical and synthesized compounds for the homologues D_2 to O_8CDF. Not all synthesized isomers have been readily available to all laboratories because of limited supplies. The situation is fast changing, but it is evident from the data that except for the tetra and octa congener(s), there remains an acute shortage. Until this situation is rectified, it is not possible to completely evaluate the relative merits of liquid phases as packed or capillary columns. Capillary columns apparently offer the best isomer resolution.

Appendix V discusses the synthesis of PCDFs in general terms and reviews the current literature. Table V-1 lists the PCDF isomers known to be isolated or synthesized as separate identities.

Table 3-2. Gas chromatograph retention times of authentic PCDFs relative to dieldrin (after Bowes *et al.* 1975b).

Structure of PCDF	Column[α]			
	OV-1	SP-2401	OV-17/QF-1	OV-1/210/225
2,3,8-	0.82	0.68	0.83	0.77
2,3,6-	0.85	0.72	0.88	0.80
2,3,4-	0.87	0.71	0.90	0.81
1,3,7,8-	1.26	0.89	1.21	1.02
1,3,6,7-	1.29	0.92	1.26	1.09
2,3,6,8-	1.42	1.10	1.74	1.28
2,4,6,7-	1.42	1.18	1.59	1.32
2,3,7,8-	1.57	1.28	1.70	1.30
2,3,6,7-	1.64	1.34	1.92	1.56
3,4,6,7-	1.68	1.47	1.96	1.64
1,2,3,7,8-	2.70	1.84	3.08	2.15
1,2,3,6,7-	2.74	1.87	3.16	2.20
2,3,4,7,8-	2.96	2.29	3.66	2.66
2,3,4,6,7-	3.04	2.39	3.80	3.01
1,2,3,6,7,8-	4.13	3.11	5.37	3.71

[α] See Table 3-1 for dimensions and operating conditions.

Table 3-3. Observed relative retention times of PCDDs and PCDFs on glass capillary columns (after Buser 1976b).

Compounds	Retention time ranges relative to O_8CDD		
	OV-101	OV-17	Silar 10C
T_4CDDs	0.13-0.18	0.10-0.15	0.12-0.22
P_5CDDs	0.20-0.27	0.16-0.24	0.18-0.30
H_6CDDs	0.32-0.41	0.28-0.38	0.31-0.48
H_7CDDs	0.56-0.63	0.52-0.60	0.54-0.62
O_8CDDs	1.000	1.000	1.000
T_4CDFs	(0.10-0.14)	(0.09-0.12)	(0.11-0.19)
P_5CDFs	0.17-0.24	0.14-0.23	0.15-0.25
H_6CDFs	0.29-0.42	0.25-0.40	0.24-0.50
H_7CDFs	0.51-0.65	0.47-0.66	0.44-0.66
O_8CDFs	0.978	1.025	0.933

Table 3-4. Relative retention times (RRT) for the 38 T_4CDFs listed in order of increasing RRT on both SP-2330 and SE-54 capillary columns (after Mazer *et al.* 1983).

T_4CDF	RRT[a] (SP-2330)	T_4CDF	RRT[a] (SE-54)
1368	0.550	1368	0.846
1378	0.620	1468	0.870
1379	0.620	2468	0.882
1347	0.630	1347	0.895
1468	0.640	1247	0.897
1247	0.647	1367	0.900
1367	0.652	1378	0.900
1348	0.666	1346	0.902
1346	0.687	1246	0.903
1248	0.690	1348	0.909
1246	0.707	1379	0.909
1268	0.707	1248	0.915
1237	0.713	1268	0.925
1478	0.717	1478	0.929
1369	0.720	1467	0.935
2349	0.754	1237	0.943
1234	0.758	1369	0.944
2468	0.763	2368	0.947
1238	0.763	2467	0.961
1467	0.769	1238	0.962
1236	0.772	1469	0.966
1349	0.800	2349	0.966
1278	0.807	1234	0.968
1267	0.847	1278	0.979
1279	0.850	1267	0.991
1469	0.861	1349	0.991
1249	0.867	1249	1.000
2368	0.867	2378	1.000
2467	0.919	1279	1.002
2347	0.963	2346	1.004
1239	0.965	2347	1.004
1269	0.998	2348	1.004
2378	1.000	2367	1.026
2348	1.011	3467	1.037
2346	1.035	1269	1.038
2367	1.050	1239	1.051
3467	1.135	1236	0.963
1289	1.210	1289	1.103

[a] RRT are relative to RT of [$^{37}Cl_4$] 2,3,7,8-T_4CDF, which by definition equals 1.000.

Table 3-5. A comparison of the number of PCDF congeners from selected matrices reported separating on some GC capillary columns and the number of congener standards reported in the literature.

Reference	Capillary Specifications[a]	Di (16)[β] [3][γ]	Tri (28) [7]	Tetra (38) [38]	Penta (28) [12]	Hexa (16) [10]	Hepta (4) [3]	Octa (1) [1]	Sample
Buser (1976b)	22 m, OV-101; 225°	-	-	-	2	5	4	1	PCP Formulation
	22 m, OV-17; 225°	-	-	-	-	4	4	1	"
	22 m, Silar 10C; 205°	-	-	-	-	5	4	1	"
Rappe et al. (1977)	22 m, OV-101; 197°	-	3	8	9	3	-	-	Yusho oil
	22 m, OV-17; 207°	-	6	9	12	3	-	-	"
Buser et al. (1978 a)	50 m, OV-17; 100-250°	+	17	21	6	-	-	-	Aroclor 1254
	50 m, OV-17; 100-250°	-	6	9	5	10	4	1	Aroclor 1260
	50 m, OV-17; 100-250°	-	18	21	17	7	4	-	Fly ash
Buser et al. (1978b)	50 m, OV-17; 100-250°	-	5	11	11	5	3	1	Yusho oil
	50 m, OV-17; 100-250°	-	8	12	15	8	-	-	Japanese PCB
Rappe et al. (1979b)	50 m, OV-17; 210-250°	-	-	2	7	4	0	-	Human liver
	50 m, OV-17; 210-250°	-	-	8	13	8	1	-	Yusho oil
Rappe et al. (1981)	50 m, OV-17; 210-250°	-	-	1	4	8	4	1	Turtle (U.S.A.)
	50 m, OV-17; 210-250°	-	-	1	3	5	2	1	Seal (Sweden)
Bell (1983)	60 m, SP-2330; 240°	-	-	3	4	4	3	1	Synthetic mixture
Mazer et al. (1983)	60 m, SP-2330; 200-250°	-	-	38	-	-	-	-	"
	30 m, SE-54; 180-275°	-	-	38	-	-	-	-	

[a] Column specifications: column length (m), liquid phase; isothermal temperature or temperature program range in °C.

[β] Number of theoretical isomers in homologous series.

[γ] Number of synthesized isomer standards reported in the literature (Table VI-1).

Mass spectroscopy (MS) is the preferred method for detecting and quantitating PCDF residues. The systems in use are the same as or similar to those reviewed for the PCDDs in NRCC (1981b). Various configurations reported in the last decade are summarized in Table 3-6. Originally, LRGC/EI/MS was used, but as the state of the art improved, HRGC has replaced the GC packed columns. As computer technology has advanced, the ability to perform multiple ion analysis has greatly improved sensitivity, quantitation and confirmation. These new techniques make it possible to detect fg to pg quantities of the PCDF residues (Buser 1980). However, electron capture detectors are still useful where residue levels in the sample are within the range of these versatile detectors (e.g. Liberti and Brocco 1982).

The application of GC/EI/MS and mass fragmentography has been described in detail by Buser (1975) for analysis of PCDF residues in chlorinated phenol formulations. Others (see Table 3-6) used different MS systems but in general observed the same patterns. Under Buser's (1975) EI conditions the fragmentation pattern consisted of M^+ and various fragment ions thereof. The general pattern for PCDFs, PCDDs, PCDPEs and PCBs are given in Figure 3-1. The mass spectral characteristics for the first ion in each chlorine cluster and higher mass range are given in Table 3-7. Fragments in the higher mass range, producing intense responses, were used to detect, quantitate and confirm the identity of their respective parent molecules. Calculation of the chlorine isotope ratios provided additional evidence as to the residues identity.

Recently, negative chemical ionization (NCI) has been used as a replacement for or adjunct to EI/MS by cooperating groups such as Dougherty et al. (1980), Kuehl et al. (1981), Rappe et al. (1981) and Stalling et al. (1982).

For aromatic polychlorides, the dominant ion reported in the NCI spectra is usually $(M\text{-}Cl + O)^-$ through the replacement reaction;

$$M + O_2^{\cdot -} \rightarrow (M - Cl + O)^- + ClO\cdot$$

The presence of oxygen as a free radical is essential.

Rappe et al. (1981), using methane as the reactant gas, selectively monitored the PCDF congener $(M+2)^-$ ions at m/e values of 306 (tetra), 340 (penta), 374 (hexa), 408 (hepta) and 442 (octa) in purified extract of turtle and grey-seal adipose tissue. Minimum detectability was said to be 0.2 pg and 0.5 pg for 2,3,7,8-T_4CDF and O_8CDF respectively. Buser (1980) reported a one- to twofold increase in sensitivity for NCI over EI/MS for some isomers.

A new technique, rapid gas chromatography-tandem mass spectrometry, for analyzing complex sample extracts for PCDF and PCDD congener residues simultaneously (Table 3-8) has been described by Sakuma et al. (1983). Twenty-five to thirty-five extracts were analyzed in an 8-hour period using manual injection and allowing 11-16 minutes

Table 3-6. Summary of some mass spectrometry systems and detection limits reported for PCDF congeners.

Reference	Instrument	GC/MS System	Spectra Scan Range[α]	Major Ions Analyzed by SID or MID					Detection Limit
				Tetra	Penta	Hexa	Hepta	Octa	
Vos et al. (1970)	Varian MAT CH_4	LRGC/EI/MS	100-450	-	-	-	-	-	1 ug
Firestone et al. (1972)	Atlas CH-4B	LRGC/EI/MS	+	-	-	-	-	-	-
Bowes et al. (1973)	GEC AEI MS 902	dp/EI/MS	50-400	-	-	-	-	-	-
Bowes et al. (1978)	GEC AEI MS 902	dp/EI/MS	50-400	-	-	-	-	-	1 ng
Curley et al. (1975)	LKB 9000	dp/EI/MS	230-360	-	-	-	-	-	-
Buser (1975)	Finnigan 1015D	LRGC/EI/MS	-	304	338	372	406	440	10 ng
Nagayama et al. (1975)	JEOL D-100	LRGC/EI/MS	200-350	-	-	-	-	-	-
Miyata et al. (1976)	Shimadzu LKB 9000	LRGC/EI/MS	100-350	-	-	-	-	-	-
Nagayama et al. (1977)	JEOL D-100	LRGC/EI/MS	200-350	-	-	-	-	-	50 pg
Miyata et al. (1977)	Shimadzu LKB 9000	LRGC/EI/MS	100-350	-	338, 340, 342	-	-	-	5 pg
Firestone (1977)	Finnigan 3300F		260-450	-	-	-	-	-	20 pg
Rappe et al. (1977)	Finnigan 1015D	HRGC/EI/MS	+	304	338	372	-	-	-
Rappe et al. (1978c)	Finnigan 4000	HRGC/EI/MS	35-460	304	338	372	406	440	10-200 ng
Kuroki and Masuda (1978)	JEOL D-100	LRGC/EI/MS	-	304	338	372	406	-	-
Rappe et al. (1979b)	Finnigan 4000	HRGC/EI/MS	35-470	304	338	372	406	-	-
Norström et al. (1979a)	Finnigan 4000	HRGC/EI/MS	35-500	-	-	-	-	-	10-50 ng
Ryan and Pilon (1980)	Varian MAT 311A	HRGC/EI/MS	-	-	-	-	-	443.7	25 pg
Cavallaro et al. (1980b)	Finnigan 3200	HRGC/EI/MS	40-200; 201-500	304	338	372	406	440	35-160 pg
Kooke et al. (1981)	HP 5984	LRGC/EI/MS	-	304	338	372	406	440	-
Rappe et al. (1981)		HRGC/NCI/MS	-	304	338	372	406	440	0.2-0.5 pg
Stalling et al. (1982)	Finnigan 4000	HRGC/NCI/MS	-	304	338	372	406	440	-
Smith et al. (1982)	Kratos MS 50	HRGC/EI/MS	+	305.8987	-	-	-	-	-
Liberti et al. (1982a)	VG 70-70F	LRGC/EI/MS	-	303.89	337.85	371.81	405.78	439.74	50-100 pg
Gizzi et al. (1982)	LKB-2091	LRGC/EI/MS	-	304, 306	338-340	372-374	406-408	442-444	-
Stalling et al. (1983)	Finnigan 4023	HRGC/NCI/MS	240-520	306	340	374	408	442	0.5 pg
Sakuma et al. (1983)	TAGA 6000	HRGC/CI MS/MS	-	see Table 3-8 for a list of congener and daughter ions					0.3-1 pg

[α] +, indicates the spectra scan range was not given.
-, indicates no spectra taken, or reported.

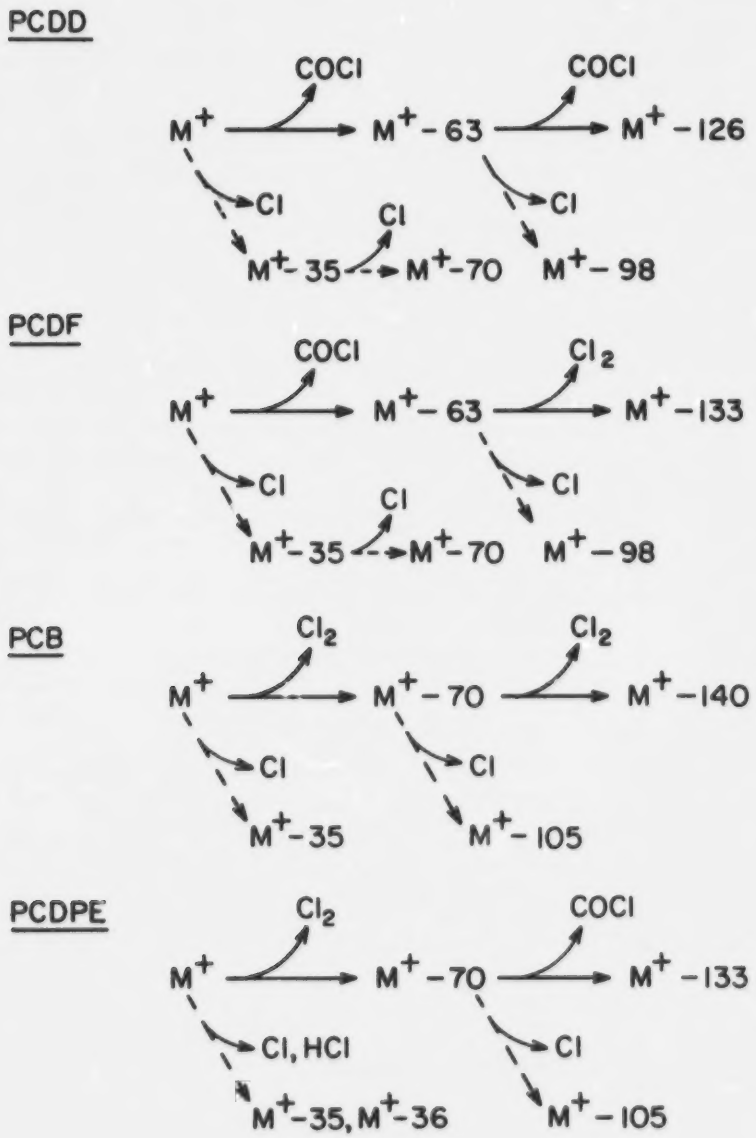


Figure 3-1. Fragmentation of PCDDs, PCDFs, PCBs and PCDPEs leading to ions in higher mass range (after Buser 1975).

Table 3-7. Mass spectral characteristics of PCDDs, PCDFs, PCDPEs and PCBs[a] (after Buser 1975).

Compound	Ion and m/e						
PCDD	M^+ (very strong)	M+ - Cl (weak)	M+ - COCl (strong)	M+ - Cl_2 (weak)	M+ - CO Cl_2 (weak)	M+ - 2COCl (medium)	M^{2+} (medium)
Tetra	320	285	257	250	222	194	160
Penta	354	319	291	284	256	228	177
Hexa	388	353	325	318	290	262	194
Hepta	422	387	359	352	324	296	211
Octa	456	421	393	386	358	330	228
PCDF	M^+ (very strong)	M^+ - Cl (weak)	M^+ - COCl (strong)	M^+ - Cl_2 (weak)	M^+ - CO Cl_2 (weak)	M^+ - COCl Cl_2 (medium)	M^{2+} (medium)
Tetra	304	269	241	234	206	171	152
Penta	338	303	275	268	240	205	169
Hexa	372	337	309	302	274	239	186
Hepta	406	371	343	336	308	273	203
Octa	440	405	377	370	342	307	220
PCDPE	M^+ (strong)	M^+ - Cl (weak)	M^+ - HCl (weak)	M^+ - Cl_2 (very strong)	M^+ - Cl_3 (weak)	M^+ - Cl_2 - COCl (medium)	M^+ - Cl_4 (weak)
Hexa	374	339	338	304	269	241	234
Hepta	408	373	372	338	303	275	268
Octa	442	407	406	372	337	309	302
Nona	476	441	440	406	371	343	336
Deca	510	475		440	405	377	370
PCB	M^+ (very strong)	M^+ - Cl (weak, med.)	M^+ - HCl (weak)	M^+ - Cl_2 (strong)	M^+ - Cl_3 (weak)	M^+ - HCl - Cl_2 (weak)	M^+ - Cl_4 (medium)
Hexa	358	323	322	288	253	252	218
Hepta	392	357	356	322	287	286	252
Octa	426	391	390	356	321	320	286
Nona	460	425	424	390	355	354	320
Deca	494	459		424	389		354

[a] Only the first ion in each chlorine cluster and in the higher mass range is listed.

Table 3-8. Ions monitored during a GC/MS/MS screen for PCDF and PCDD congeners (after Sakuma *et al.* 1983).

Congener	Parent Ion/Daughter Ion	Time Window (minutes)
M_1 CDD	218/155	0:00-3:36
M_1 CDF	202/139	
D_2 CDD	252/189	3:36-4:30
D_2 CDF	236/173	
T_3 CDD	286/223	4:30-5:12
T_3 CDF	270/207	
T_4 CDD	320/257	5:12-6:00
T_4 CDF	304/241	
T_4 CDD	332/269	
P_5 CDD	356/293	6:00-6:50
P_5 CDF	340/277	
H_6 CDD	390/327	6:50-7:28
H_6 CDF	374/311	
H_7 CDD	424/361	7:28-8:04
H_7 CDF	408/345	
O_8 CDD	460/397	8:04-9:00
O_8 CDF	444/381	
O_8 CDD	472/408	

between injections. A 15-metre DB-5 capillary column with an internal diameter of 0.32 mm is interfaced with the Townsend discharge chemical ionization (80% N, 20% O_2 reagent gas) source of a triple quadrupole instrument. This soft ionization method generates mainly molecular ions, with very little fragmentation. The first mass analyzer (Q1) selects the molecular PCDF and PCDD ions that subsequently enter a collision region, Q2 (RF only), and are fragmented when they pass through a screen of argon atoms. Under these conditions, the daughter ion fragments $[M- COCl]^+$ predominate for PCDFs and $[M-2COCl]^+$ for PCDDs. The second mass analyzer, Q3, selects the $[M-COCl]^+$ fragment ions for detection. The detection limits are 0.3 to 0.6 pg for the lower chlorinated congeners and 0.5 to 1 pg for O_8CDF and O_8CDD. The authors analyzed extracts of soil, water, fly ash, fish and human adipose tissue. This unique MS/MS configuration offers a rapid turnover of a large number of samples with minimal cleanup, excellent sensitivity and good specificity -- prerequisites for a rapid screening method.

PCDF isomers or congener groups are quantified by comparing the peak height(s) response of the molecular and/or fragmentary ions with that produced by an authentic standard. When possible, isotope-labelled standards are used to fortify similar sample matrices and recovery corrections are applied. However, as previously noted, the availability of standards is limited. When standards are not available, it is the custom to assume equivalent response of isomers in the same congener group. Interpolation between homologues has also been reported; e.g., Rappe et al. (1981) used tetra and octa PCDF standards to estimate the concentration of observed penta, hexa and hepta congeners.

3.3 INTERFERENCES AND CONFIRMATION

Interference to analysis can occur from inadequate sample cleanup, the presence of related xenobiotics, PCDF (or PCDD) precursors, contaminated equipment and the formation of artifacts under some analytical conditions. Cleanup procedures must be rigorous but not excessive to a degree that recoveries are adversely affected. Strict adherence to a sound, comprehensive laboratory security and cleaning program is essential to avoid contamination of the premises, glassware, equipment and personnel from ubiquitous compounds in samples in the laboratory environment, e.g. PCBs.

The procedures essential to removing sample matrix interferences, e.g. lipid components, are standard, well-established techniques that were comprehensively reviewed for some PCDFs in the PCDD critique (NRCC 1981b). Recently reported systems on extraction and cleanup are reviewed and rated in Appendices I and II.

Xenobiotic residues that interfere with PCDF analysis are primarily polychlorinated aromatic compounds, many of which are ubiquitous in the environment. Some, such as the PCBs, can be present

at concentrations greatly in excess of the PCDF residue. They must be removed to a level at which the analyte/interference ratio is favorable for reliable measurement by the detection component of the method system, usually HRGC/MS. Other xenobiotics of concern include the neutral polychlorinated benzenes (PCBzs), polychlorinated diphenyl ethers (PCDPEs), polychlorinated naphthalenes (PCNs), polychlorinated terphenyls (PCTs) and polychlorinated anisoles.

Removal of the neutral compounds such as PCBzs, PCBs or PCDPEs can be accomplished by separation and collection of the appropriate eluate fraction from an alumina column. Minor amounts of some PCBs, PCDPEs, PCNs and PCTs may be present in the eluate fraction containing the PCDFs and PCDDs.

Other effective procedures for removing or reducing the level of the neutral and acidic polychlorinated aromatic compounds utilize a Florisil micro chromatography/HPLC system (e.g. Ryan and Pilon 1980) or combinations of different adsorbent columns consisting of acid- and base-treated neutral-silica gels, activated carbon and alumina as described by Stalling et al. (1980, 1982a).

Accurate identification and confirmation require MS analysis and when isomers overlap on the particular HRGC column in use, a second column of different resolution pattern must be used (Rappe et al. 1983b). To differentiate between PCDDs and PCDFs, the same authors reported that separation by isomer-specific response ratios, NCI/EI, or fragmentation ratios was feasible. If a multitude of PCDDs and PCDFs were present, then chlorine-isotope cluster analysis was used as a basis for differentiating between the numerous isomers.

To confirm the identity of PCDF residues in the presence of PCNs during the analysis of fly ash and PCBs, Buser et al. (1978a) observed the complete PCDF positive-ion mass spectra (M^+), their chlorine isotope abundance, and the typical fragmentation with formation of M^+-COCl for PCDFs and M^+-Cl_2 for the PCNs. In a report concerning the analysis of Yusho oil and Japanese waste PCBs, Buser et al. (1978c) observed that the PCN hexa CN:M^++6 = 338 was not a problem in determining penta CDF:M^+ = 338 as the two compounds were separated on a 50 m OV-17 capillary column.

Smith and Johnson (1983) evaluated and categorized the interferences from seven series of polychlorinated aromatic compounds in GC/MS analysis (see Table 3-9). Note that the diphenyl ethers, methoxy and hydroxy PCBs give fragments which are indistinguishable from those produced from PCDFs. The authors indicated that in their experience, the presence of these contaminants at 1 part in 10^4 of analyte could seriously compromise the validity of HRGC/MS-MID as well as HRGC/HRMS-MID analysis, if not removed in the cleanup.

The type of data produced using the newer, more reliable analytical techniques suggests that the accuracy of older data must be

Table 3-9. Levels of interference of selected chemical families in MS determinations of PCDFs and PCDDs (after Smith and Johnson 1983).

Family of polychlorinated compounds[γ]	Level of Interference					
	Overlap of fragmentation patterns[α]		Indistinguishable by LRMS[β]		Indistinguishable by HRMS[β]	
	PCDDs	PCDFs	PCDDs	PCDFs	PCDDs	PCDFs
PCBs	++/+++					
PCNs		+				
DPEs		++/+++		X		X
MeO-PCBs	+++	+++	X	X		X
HO-PCBs		+++		X		X
MeO-DPEs	+++	++	X		X	
HO-DPEs	+++		X		X	
BzPEs	+++		X			
Biphenylenes	++					

[α] In the first two columns "+" indicates minor overlap, "++" indicates major overlap, "+++" indicates complete overlap.

[β] In the last four columns, an "X" indicates that interference occurs due to one of the polychlorinated compounds.

[γ] The abbreviations used for polychlorinated compounds are: PCBs-biphenyls; DPE-diphenyl ethers; PCNs-napthalenes; BzPEs-benzylphenyl ethers. The prefixes MeO- designate methoxy and HO-hydroxy.

suspect. Even today, it is difficult to avoid the influence of artifacts and other interferences on the accuracy of analysis at or below $ng.kg^{-1}$ levels.

3.4 SAMPLING, EXTRACTION AND CLEANUP

3.4.1 INTRODUCTION

This section reviews procedures for the sampling, extraction, and cleanup of sample matrices. Each report has been reviewed in detail and the information summarized on an individual basis in Appendices II, III and IV. The rating system fully described in Section 2.1 of NRCC (1981b) has been used to evaluate the methods as reported. A summary of the rating criteria are given in Appendix I.

3.4.2 BIOLOGICAL MATRICES

The method summaries are to be found in Appendix II of this volume or, where previously reviewed, in NRCC (1981b). For the reader's convenience, Table 3-10 lists the matrices with reference sources.

It has not been possible to evaluate sampling procedures *per se*, as none of the papers reviewed provided sufficient detail in this important and crucial aspect of analytical chemistry. Extraction solvents and techniques are summarized in Table 3-11. The data indicate that organic solvents such as hexane, cyclohexane, methylene chloride, chloroform, acetonitrile, methanol and combinations thereof are extensively used. Various extraction techniques are employed. The relative extraction efficiency of one solvent or technique over another has not been determined for biological matrices or a variety of PCDFs.

Lipids are separated from the sample extract by a variety of techniques such as those listed in Table 3-12. They range through saponification, concentrated acid treatment, acetonitrile partitioning to gel permeation using Bio beads. The relative merit of one system over another has never been adequately evaluated. Firestone (1977) reported a substantial loss (> 60%) of O_8CDF during EtOH/KOH extraction of fortified gelatin samples, but excellent recovery (89%) of 2,3,7,8-T_4CDF. Conversely, in a controlled experiment involving PCDF fortified vegetable oil containing PCB, Nagayama *et al.* (1976) reported excellent recoveries of tetra and penta homologues following saponification under their conditions.

Loss of the lower chlorinated M_1CDF, D_2CDFs and T_3CDFs, was observed during concentrated H_2SO_4 cleanup of fly ash extracts (Sakuma and Ryan 1983). Whether simfliar loss would occur with extracts of plant and animal tissues has not been ascertained. Considering this

Table 3-10. A listing of biological sample matrices by origin.[a]

Matrix	Reference
ANIMAL	
turtle, seal	Rappe et al. (1981)
chicken liver	Ryan and Pilon (1980)
salmon oil	Stalling et al. (1980)
milk, blood, feces, fat	Firestone et al. (1979)
human tissue	Rappe et al. (1979b)
"	Kuroki and Masuda (1978)
"	Miyata et al. (1977)
"	Nagayama et al. (1977)
"	Ryan and Williams (1983)
gelatin	Firestone (1977)
fish	Stalling et al. (1979b, 1983)
"	Kuehl et al. (1981)
blood	Rappe et al. (1983d)
PLANT	
wood shavings	Ryan and Pilon (1980)
rice oil	Rappe et al. (1977)
Yusho oil	Nagayama et al. (1976)
corn oil	Williams and Blanchfield (1972)
wood dust	Levin and Nilsson (1977)
OTHER	
monkey feed	Farrell (1980)
aquatic origin	Stalling et al. (1980, 1982)
river sediments	Petty et al. (1982)

[a] Details in Appendix I of NRCC (1981b) or in Appendix II of this monograph.

Table 3-11. A summary of extraction techniques and solvents reported for biological matrices.[γ]

Matrix	Technique	Solvent	Reference
human tissue	homogenized	n-hexane	Nagayama et al. (1977)
"	"	"	Kuroki and Masuda (1978)
"	"	"	Rappe et al. (1979b)
"	reflux 1 h	EtOH/KOH	Miyata et al. (1977)
"	shake	hexane/acetone	Ryan and Williams (1983)
commercial gelatin	shake 1.5-2 h	"	Firestone et al. (1979)
gull eggs	Soxhlet 8 h	hexane/acetone	Bowes et al. (1973)
sea lion adipose	" 4 d	"	"
" liver[α]	" 72 h	"	"
fish tissue	" 4 h	"	Dougherty et al. (1980)
"	" 8 h	"	Kuehl et al. (1981)
"	column chromatography	CH_2Cl_2	Stalling et al. (1979a)
"	"	"	Dougherty et al. (1980)
"	"	C_6H_{12}/CH_2Cl_2	Smith (1980)
"	"	"	Stalling et al. (1982) (1983)
"	"	"	
turtle, seal tissue	"	"	Rappe et al. (1981)
chicken liver	homogenized	$CHCl_3/CH_3OH$	Ryan and Pilon (1980)
rice oils[β]	partition	acetonitrile	Bowes et al. (1975a)
"	reflux	EtOH/NaOH	Nagayama et al. (1975, 1976)
"	"	"	Rappe et al. (1977)

[α] Sea lion livers were freeze dried 5 days prior to extraction.

[β] Rice oils as described here include the PCB contaminated "Yusho oils".

[γ] See Appendix II for details.

Table 3-12. A summary of some clean-up procedures to separate lipid compounds from biological extracts.[a]

Matrix	Extract	Clean-up	Reference
human tissue	hexane	saponification with EtOH/KOH	Nagayama et al. (1977)
"	"	"	Kuroki and Masuda (1978)
"	"	"	Rappe et al. (1979b)
"	hexane/acetone	H_2SO_4 partition	Ryan and Williams (1983)
rice oil	no preliminary extract	acetonitrile partition	Bowes et al. (1975a)
corn oil	"	H_2SO_4 partition	Williams and Blanchfield (1972)
rice oil	"	chromatography on silica gel	Buser et al. (1978c)
fish oil	CH_2Cl_2	gel permeation complex series of base/acid/ carbon/glass fiber columns	Stalling et al. (1979), (1980)
"	"	"	"
chicken liver	$CHCl_3/CH_3OH$	hexane/ H_2SO_4 partition	Ryan and Pilon (1980)
sea gull eggs	hexane/acetone	celite/H_2SO_4 filter pad	Bowes et al. (1973)
sea lion liver	"	"	"
sea lion adipose	"	"	"

[a] See Appendix II for details.

report and the controversial observations with respect to saponification there is a need for interlaboratory validation studies of these widely used clean-up methods.

A unique but complex system that incorporates features amenable to automation has been described by Stalling et al. (1979b) for most xenobiotic residues. The schematic of the original concept is given here (Figure 3-2). Certain modifications have been made for PCDF and PCDD analysis over the intervening years by various members of the Columbia National Fisheries Research Laboratories. The new flow diagrams for enrichment and fractionation are reproduced here as Figures 3-3 and 3-4 respectively (Stalling et al. 1982). Smith (1981) described the development and use of the carbon/glass fibre adsorbent that is superior in performance to the carbon/polyurethane foam previously used. Dougherty et al. (1980) preceded the carbon/polyurethane foam column with gel-permeation chromatography (GPC) to remove lipid material. The use of GPC had been previously reported by Kuehl and Leonard (1978).

The modified system of Stalling et al. (1982) has undergone a comprehensive evaluation using fish tissues fortified with ^{13}C-T_4CDD and non-labelled PCDF standards (Stalling et al. 1983). The authors report 50-75% recovery of the PCDF at an unspecified fortification level, 60 to 90% of the ^{13}C-T_4CDD at 20 $ng.kg^{-1}$ and 80% at 100 $ng.kg^{-1}$. On the basis of 20 g of original fish tissue, the residue remaining in the final analyte concentrate has been reported as approximately 200 ng. This procedure received one of the highest ratings when evaluated against several different T_4CDD clean-up procedures (Brumley et al. 1981).

Other clean-up procedures (e.g. Nagayama et al. 1977) are not as involved. The hexane portion from the liquid/liquid partition of the aqueous saponification mixture is concentrated and passed through a silica-gel column followed by fractionation on an alumina column. Miyata et al. (1977) used a similar procedure but substituted Florisil for the silica-gel adsorbent step. Ryan and Pilon (1980) followed their concentrated-acid hexane partition with a Florisil column to remove PCBs, then fractionated the remaining residues on a HPLC column. Ryan and Pilon (1982) reported on the use of polyaromatic hydrocarbons as secondary standards for calibrating the HPLC column.

The remaining procedures, summarized in Appendix II for biological matrices, are similar in many respects to those discussed previously.

Except for the works of Brumley et al. (1981) and Ryan et al. (1983) involving TCDD residues, there are no reports that validation studies for PCDF methods have been organized or carried out.

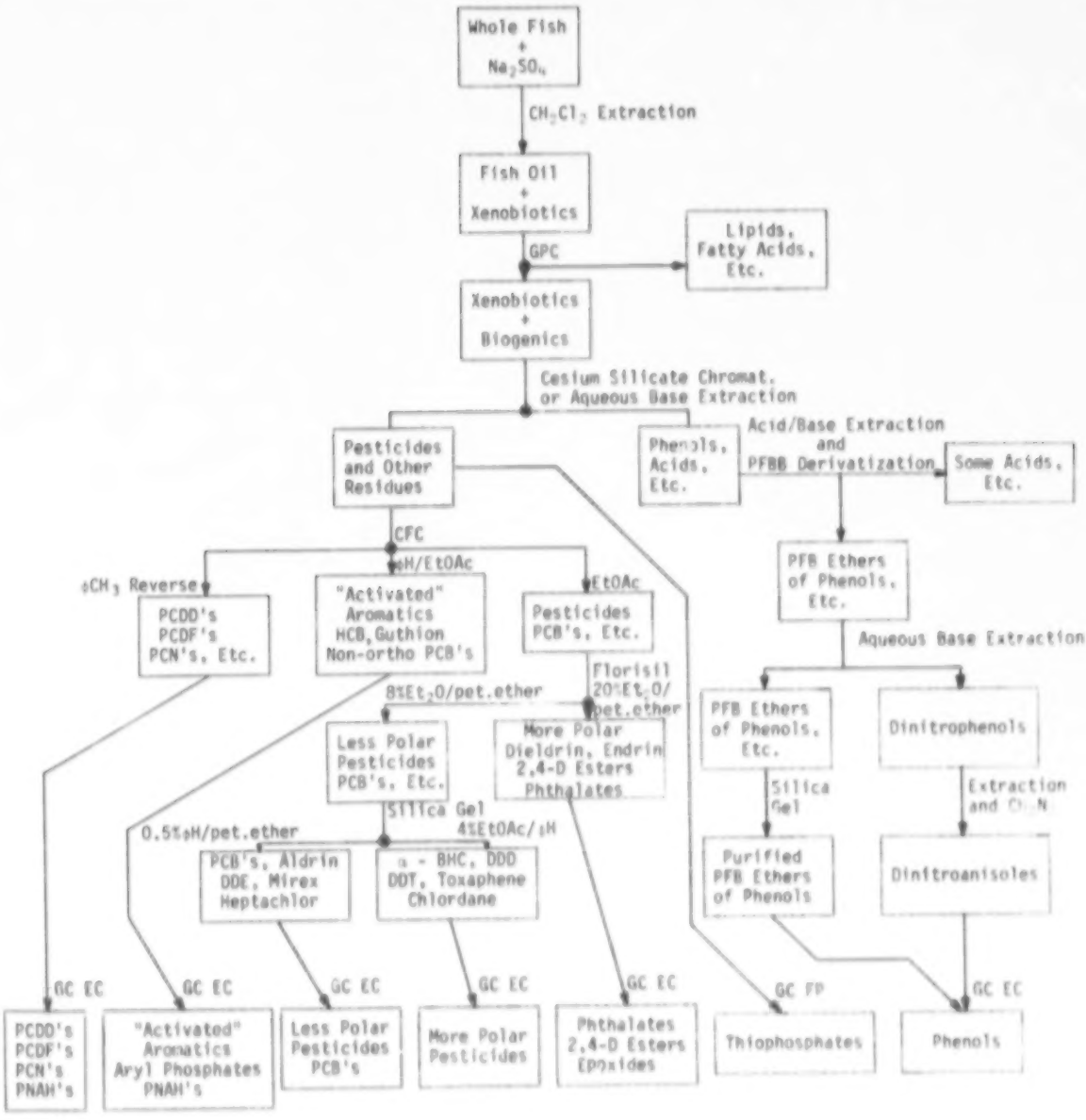


Figure 3-2. A generalized scheme for multiclass chemical residue cleanup and determination (from Stalling et al. 1979b; Copyright, ASTM, 1916 Race Street, Philadelphia, PA. 19103. Reprinted with permission.).

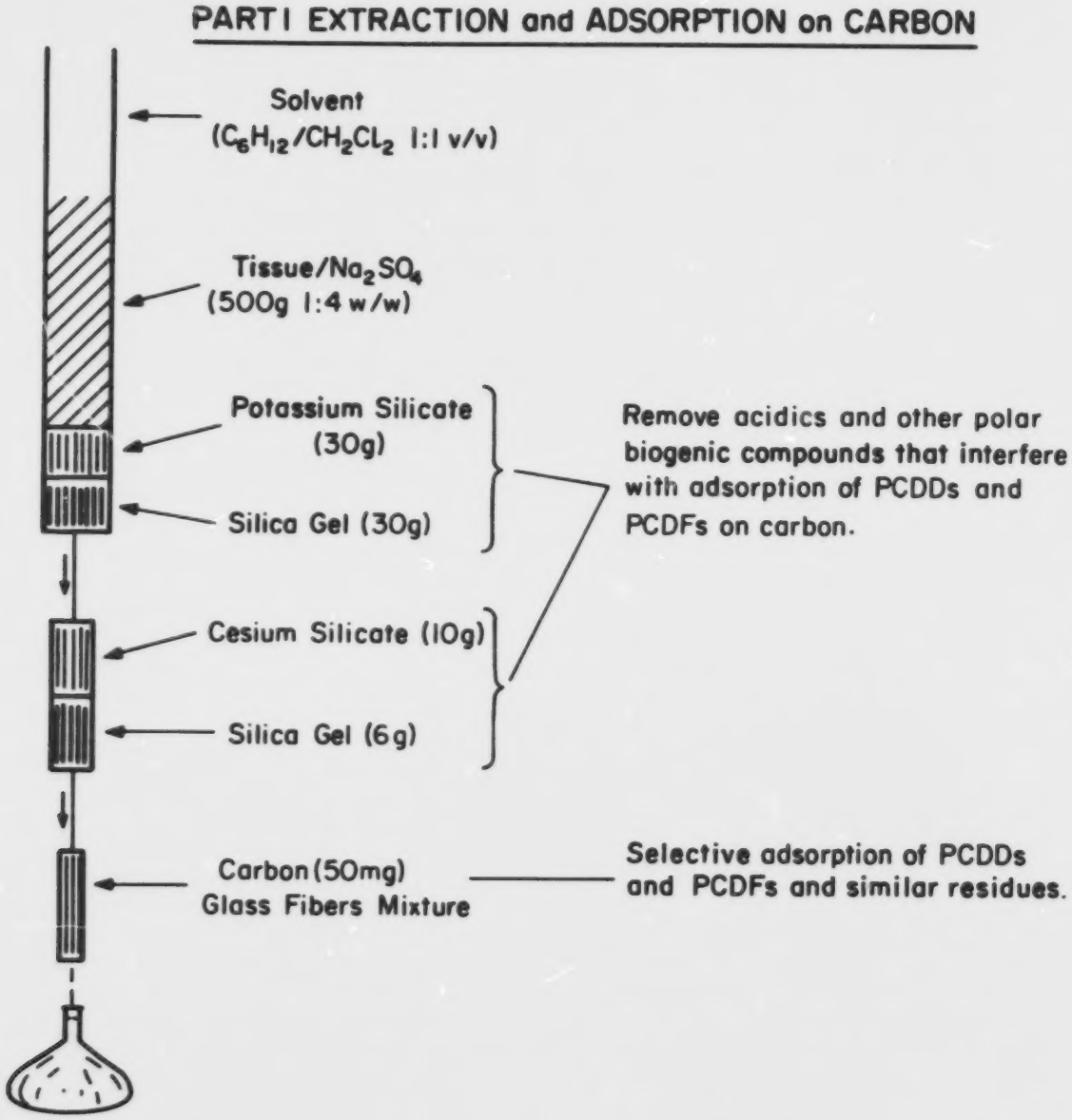

PART I EXTRACTION and ADSORPTION on CARBON
Solvent
(C_6H_{12}/CH_2Cl_2 1:1 v/v)
Tissue/Na_2SO_4
(500g 1:4 w/w)
Potassium Silicate
(30g)
Silica Gel (30g)
Remove acidics and other polar biogenic compounds that interfere with adsorption of PCDDs and PCDFs on carbon.
Cesium Silicate (10g)
Silica Gel (6g)
Carbon (50mg)
Glass Fibers Mixture
Selective adsorption of PCDDs and PCDFs and similar residues.

Figure 3-3. Flow diagram for enrichment of PCDDs and PCDFs (Stalling et al. 1982; reprinted with permission from Chlorinated Dioxins and Related Compounds. Impact on the Environment, copyright 1982, Pergamon Press.).

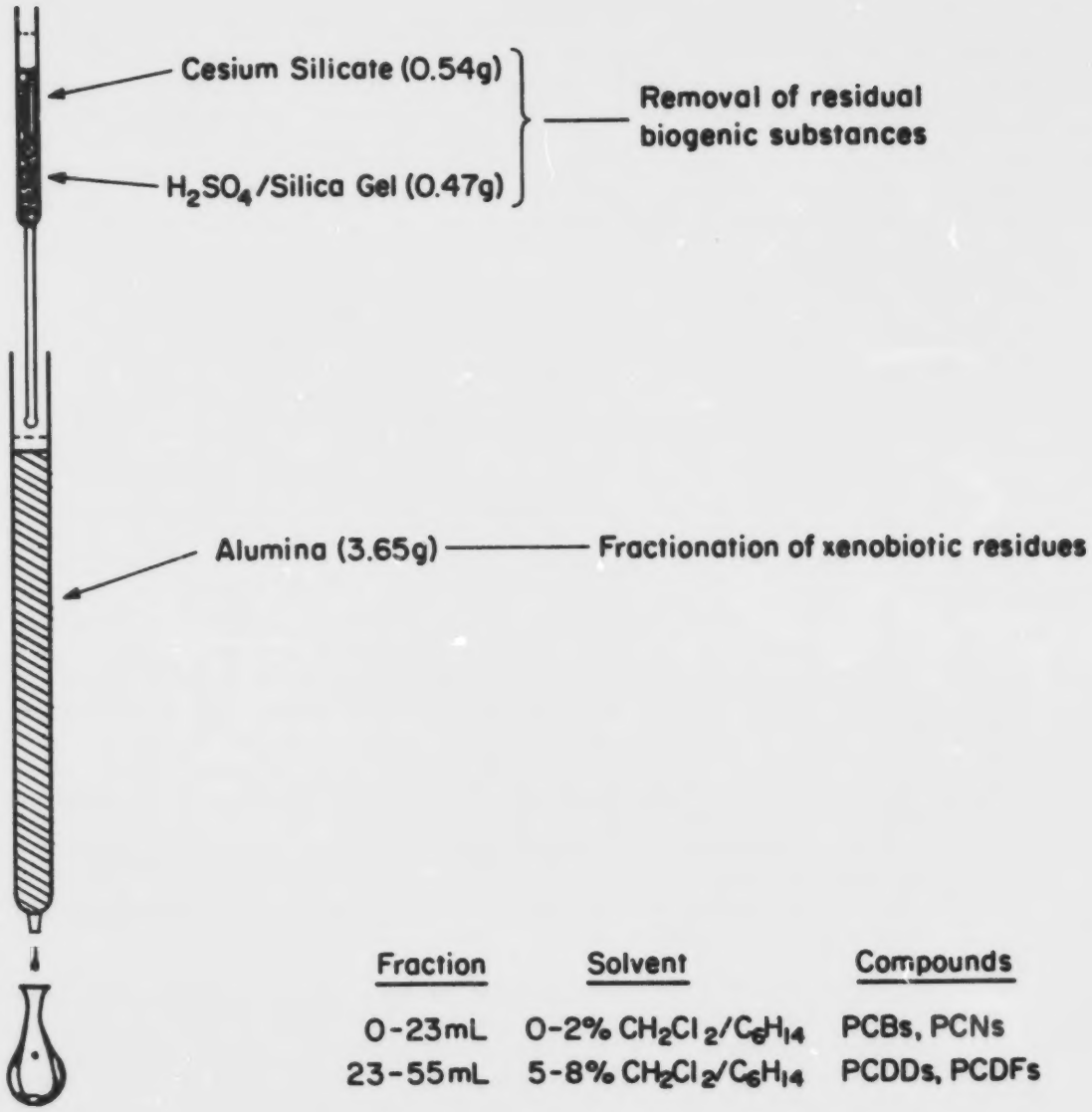


Figure 3-4. Flow diagram for fractionation of PCDDs and PCDFs (Stalling et al. 1982; reprinted with permission from Chlorinated Dioxins and Related Compounds. Impact on the Environment, copyright 1982, Pergamon Press.).

3.4.3 COMBUSTION SOURCES

There have been no major changes in the basic approach to sampling, extracting and clean-up procedures for slag, quench water, fly ash, soot, coal, flue gases, refuse fuels, etc. since the critique on the limitations to PCDD analysis (NRCC 1981b) was published. The few current reports related to PCDF methods are listed in Table 3-13 and their contents are summarized in Appendix II. For convenience, those reports directly concerned with PCDF methodology and reviewed in NRCC (1981b) are also listed with reference to the page and table numbers of that publication.

A comprehensive method manual from the United States Environmental Protection Agency (USEPA) was published in January 1982 (Stanley et al. 1982). These guidelines document in detail the sampling and analysis approach for assessing stationary combustion source emissions in the U.S. mid-west. They can be considered an excellent starting point for establishing standard practices for the quantitative and qualitative analysis of organic emissions. The authors provide background on the methodology recommended but refer the reader to other USEPA publications for explicit details on some elements of the methodology (USEPA 1976; Adams et al. 1977).

Despite the availability of documented sampling methodology (USEPA 1971; Cavallaro et al. 1980a; Stalling et al. 1982) much remains in the developmental state and considerable effort is required to improve and validate the procedures. Specifically, it is necessary to delineate for all sample types:

1) the relation between particle size, temperature and the need to sample various matrices;
2) the effectiveness of various approaches for the homogenization of samples; and
3) the effectiveness of the various approaches being used to obtain representative samples.

Soxhlet refluxing continues to be favored as the technique for extracting residues from solids despite the fact that Kimble and Gross (1980) noted a discrepancy in an evaluation report by Lustenhower et al. (1980) and went on to cite data showing the ultrasonic probe to be more efficient than the ultrasonic bath or Soxhlet refluxing. The latter technique did not always provide extractant penetration to all areas of the sample mass, as indicated by dry areas at the end of the extraction times used.

Kooke et al. (1981) compared seven methods for extracting PCDD and PCDF residues from fly ash and noted wide variation in extraction efficiency. Soxhlet extraction with benzene or toluene following acid treatment of the ash gave the best and most consistent results. Solvents such as methylene chloride, chloroform and acetone/hexane were less efficient as were methods using shaking or ultrasonic treatment.

Table 3-13. A list of references related to PCDF residue analysis of combustion source matrices.

Original Reference	Summary Table No. and Page
	This Document
Stanley et al. (1982)	IV-1
Liberti and Brocco (1982)	
Liberti et al. (1982a, b)	
Gizzi et al. (1982)	
Rappe et al. (1982)	
	NRCC (1981b)
Kooke et al. (1981)	II-5
Cavallaro et al. (1980a)	II-3
Lustenhower et al. (1980)	II-4
Eiceman et al. (1979)	II-8
Buser et al. (1978a)	II-9
Olie and Hutzinger (1978)	II-11
Olie et al. (1977)	II-11

Recently, Liberti et al. (1982a) compared the PCDF and PCDD residue extraction efficiencies of fourteen combinations made up from four different methods, three solvents, four different times, and acid and base treatments from particulate matter of a fly ash sample. The cold, leaching process using C_6H_6-CH_2Cl_2 was superior overall.

It is still not known what constitutes the "ideal solvent". Uncertainties about the extraction efficiency of the techniques used today arise because of our poor understanding of how PCDFs (and PCDDs) are incorporated into the particle during combustion. While the adsorbed residues on the surface are probably removed, those incorporated in or bound to the inner matrix remain. Thus, present data on residue levels will invariably represent minimum values and should be considered as estimates, not actual values.

Procedures and apparatus for trapping gaseous emissions and condensates are described in detail by USEPA (1976) and Stanley et al. (1982). Gizzi et al. (1982) illustrated a three-step trapping system used to collect compounds in the vapor phase. The trapping solvent is generally diethylene glycol, with hexane or methylene chloride as the partitioning solvents for the recovery of residues from this and other aqueous condensates or liquids such as quench water.

Clean-up procedures reported for extracts of combustion range from the multi-column chromatography system described by Cavallaro et al. (1980a) to a simple extraction, no chromatography procedure described by Olie et al. (1977) or Eiceman et al. (1979). Between these extremes, a silica separation followed by alumina column fractionation has been found to provide an analyte concentrate suitable for HRGC/MS or EC analysis (Buser et al. 1978a; Liberti et al. 1982a). Multi-column chromatography, or its equivalent, is essential if interference to PCDF residue determination is to be avoided. Most of the methods lack the validation data that support their reliability to produce accurate results.

3.4.4 PESTICIDE FORMULATIONS AND OTHER CHEMICAL PRODUCTS

The references on PCDF methodology for pesticide formulations and other chemical products, e.g. PCBs, are listed in Table 3-14. Seven studies have been previously reviewed and summarized in NRCC (1981b). Five reports were not previously reviewed as they were concerned primarily with PCDF residues; summaries of their contents are in Appendix III of this volume.

Formulations are diluted mixtures of the technical concentrate and may be in liquid, granular or powder form. They contain emulsifiers, wetting agents, solvents and unknown substances in various preparations and combinations. A given formulation can vary widely from one production lot to another and between manufacturers. This situation

Table 3-14. A list of references to PCDF analysis of pesticide formulations and other chemical products.

Matrix	Original Reference	Details in Table No.
		(a) This Document
Phenoxyalkanoic acids	Baker et al. (1981)	III-1
PCPs	Rappe et al. (1978a)	
Kaneclors (PCBs)	Miyata et al. (1976)	
Aroclors and Kaneclors	Bowes et al. (1975b)	
Aroclor 1254	Curley et al. (1975)	
		(b) NRCC (1981b), Appendix III
PCPs	Buser (1975)	A.8
"	Buser and Bosshardt (1976)	A.6
Kaneclors 300-600	Masuda et al. (1976)	C.2
2,4,5-T	Buser et al. (1978a)	B.7
2,4-D, 2,4,5-T	Norström et al. (1979a)	B.2
2,4,5-T, Herbicide Orange	Rappe et al. (1978a)	B.7

makes it difficult for a given method to be satisfactory for all lots or batches of the same formulation.

Technical concentrates of agricultural chemicals do not have the multiplicity of ingredients that their formulations do, but reaction products, such as PCDF residues, require extensive cleanup to be analyzed.

The sampling, extraction and clean-up procedures for PCDFs and PCDDs are essentially the same. The authors are not aware of any significant advancement or changes since the NRCC (1981b) document was published. Consequently, the critique (Appendix VI), the tables of reference material and the comments in NRCC (1981b) are current and applicable to the elements of PCDF methodology under discussion. Combining that information with the comments and observations made by Baker et al. (1981) leaves little to add. Although Baker et al. (1981) restricted their review to methods for PCDF residues in chlorophenoxyalkanoic acid herbicides their critique also applies to other pesticide and chemical products. There is a need to publish explicit details of procedures and validation studies.

4.0 ENVIRONMENTAL PATTERNS AND PERSISTENCE

Very little has been published that is useful for evaluating the behavior of PCDFs in the environment. To gain even a qualitative concept of the behavior of the PCDFs in most environmental situations, we must extrapolate from the limited data pertinent to the PCDDs. Although the two categories of compounds are expected to be somewhat parallel, such extrapolations are of limited usefulness. As reviewed in NRCC (1981a), most data on the PCDDs relate only to 2,3,7,8-T_4CDD and even then the studies are "generally poor, quantitative interpretations are frequently absent and extrapolations from situation to situation are usually impossible". Furthermore, as will be discussed, differences in the partitioning patterns, and possibly degradation patterns, of isomers complicate any interpretation of residue data.

The few published data pertinent to the environmental behavior of the PCDFs are discussed below. A minimum data base for even a preliminary analysis of the environmental behavior of a PCDF isomer would include:

(1) measures of its partitioning potential into hydrophobic environmental compartments (e.g. the octanol:water partition coefficient (K_{ow}) or the water solubility); and

(2) information on the various operative degradative processes. If basic information (e.g. the quantum yield and absorbance spectrum, and nature of the process for photolysis) is available, then the likely rate of degradation in specific situations can be predicted. It is also important to characterize toxic degradation products.

4.1 PERSISTENCE DATA

4.1.1 PHOTOLYSIS

PCDFs are known to be formed by the photoreaction of certain chemicals (see Chapter 2). However, there is some evidence that they can also be broken down by photolytic processes. A critical question, so far unanswered, is whether the more highly chlorinated PCDFs can be photodegraded to increase the relative amount of highly toxic congeners. The quantum yield and absorbance spectra are generally not available for PCDF congeners.

Photodegradation of a chemical using artificial light in the laboratory does not necessarily imply that the process will occur at a significant rate under natural sunlight (>280 nm) in a given environment. Some of the variables to be considered include the role of sensitizers and quenchers, hydrogen donors, competing nucleophiles and "sunscreens" that occur in the field (Bunce *et al.* 1978; Crosby *et al.* 1973).

Crosby et al. (1973) reported that 2,8-D_2CDF was photodegraded to a relatively stable 2-M_1CDF, under an "environmentally relevant" fluorescent UV lamp (300-450 nm), in laboratory grade methanol; the half-life was about 30 h. However, the inadvertent use of highly purified methanol in the same experiment revealed much slower photolysis ($t_{\frac{1}{2}}$ ≈ 100 h). The addition of acetone to the purified methanol did not sensitize the photolysis, but the addition of 6,6'-dichlorobenzophenone resulted in a sharp increase in the rate. The photolytic degradation of at least 2,8-D_2CDF is obviously very dependent on the "impurities" present in the experimental solution. In the case of the PCDDs, the presence of a hydrogen donor apparently is essential (NRCC 1981a). Crosby and Moilanen (1973) state that 2,8-D_2CDF was photodegraded only slowly in aqueous suspension.

Hutzinger et al. (1973) reported that 2,8-D_2CDF and O_8CDF were photodegraded very rapidly when irradiated by a fluorescent UV lamp (310 nm) in methanol ($t_{\frac{1}{2}}$ for O_8CDF < 30 min) or hexane ($t_{\frac{1}{2}}$ for O_8CDF and D_2CDF < 2 h). 2,8-D_2CDF yielded a T_3CDF as well as a monochloro derivative. However, degradation in bright sunlight was relatively slow (no rate given) when the PCDFs were exposed as thin films on the inside of quartz tubes.

Mazer and Hileman (1982) reported that when various T_4CDF isomers were exposed to a 254 nm UV light source, there was an initial loss of one chlorine atom. There was a specific order of reactivity for the Cls:

i) Chlorines on the aromatic ring tend to hinder the loss of Cl from that ring;

ii) Vicinal Cls hinder the loss of the associated Cl; and

iii) Given an equal number of vicinal Cls, the 2-Cl will be lost before the 3-Cl.

It must be emphasized that irradiation at 254 nm has little bearing on what may happen in the environment, since sunlight is filtered out below about 290 nm. Mazer and Hileman (1982) point out the possible use of this experiment in identifying T_4CDFs or in synthesizing T_3CDFs.

4.1.2 BACTERIAL AND FUNGAL CATABOLISM

In general, bacterial processes are the most ill-defined of those pertinent to a pollutant's fate. There are no published data on the PCDFs. The PCDDs, specifically T_4CDD, have been reported to be highly resistent to bacterial catabolism (NRCC 1981a), although it was speculated that the lesser chlorinated homologues may be more susceptible to degradation, as was the case with PCBs (Roberts et al. 1978). Similar speculations could be made for the PCDFs.

Cerniglia et al. (1979) found that non-chlorinated dibenzofuran was oxidized by a bacterium (Beijerinckia B8/36) and a fungus (Cunninghamella elegans) to 2,3-dihydroxy-2,3-dihydrodibenzofuran.

4.1.3 VOLATILIZATION

The potential importance of volatilization as an effective process for the PCDFs cannot be predicted because the solubilities and vapor pressures have not been accurately determined for any of the congeners. Based on the PCDDs (NRCC 1981a), the volatilization rate constants would be expected to be quite slow (order of days cm^{-1}), but the process could still be relatively important to environmental distribution.

4.2 PREDICTION AND VERIFICATION OF PARAMETERS RELEVANT TO POLLUTANT FATE

As discussed by Roberts et al. (1981) it is usually possible to develop some gross initial predictions of how a chemical will behave in a given situation from a few physicochemical parameters including the molecular weight, vapor pressure, quantum yield, UV absorbance spectra, octanol:water partitition coefficient and biodegradation potential. A computer model was developed to facilitate the process. Roberts and Boddington (1983) used the model to make some general predictions about the behavior of the PCDDs in mock aquatic systems. This exercise could be repeated for the PCDFs but the paucity of information on both classes of compound is such that similar assumptions would be made, and hence similar conclusions drawn, with the same lack of confidence.

NRCC (1981a) examined the predicted dynamics for 2,3,7,8-T_4CDD continuously input to mock aquatic systems until equilibrium, followed by the cessation of input and subsequent clearance. The results showed that the sinks were the sediments and the suspended solids so that the size of these pools would have a dramatic effect on the persistence. Volatilization was predicted to be the main removal (redistribution) process but the calculations suggested that even a low microbial degradation rate constant could have a significant impact. This may simply reflect the lack of information on relevant processes. Clearly, sediments and suspended solids may be the best monitoring matrices for PCDD or PCDFs. It is pointed out (NRCC 1981a) that fish may make the best initial indicators because levels in fish usually reach equilibrium faster than levels in the sediments. Levels in fish also give some indication of bioavailability.

Because the PCDDs and PCDFs display a range of solubilities or octanol:water partition coefficients (K_{ow}) (range for the PCDDs is through 5 orders of magnitude rising with the degree of chlorination,

NRCC 1981a), they will "sink" to varying extents into the organic matrices, even though they are all quite hydrophobic (e.g. K_{ow} for 2,3,7,8-T_4CDD = 1.4 x 10^6; NRCC 1981a). Thus, when a PCDF mixture enters an ecosystem, the homologue pattern should differ between matrices (as long as sorption is the dominant process and the sorptive capacity of the medium is not exceeded). Extrapolating from the predictions for the PCDDs (Roberts and Boddington 1983) the relative amount of the more highly halogenated PCDFs would be expected to be higher in organic matrices than in the source material. This was, in fact, observed in Woods Pond, MA (see Table 2-25). A similar shift would be expected in the homologue patterns of residues in fish tissues (again assuming that saturation does not occur). The tissue accumulation patterns for the PCDFs (see Chapter 6) does in fact change with such subtle changes in structure that metabolic processes as well as solubility are likely involved.

This means, of course, that caution must be exercised when interpreting data from various matrices. The partitioning characteristics of the isomers must be known in order to make estimations. Added complications will arise if certain homologues degrade at different rates. Relevant studies do not exist for either the PCDDs or PCDFs. The authors of NRCC (1981a) pointed out that the end use of data best decides the choice of indicator matrix and that only the most general conclusions or extrapolations can be drawn from any residue data.

Nevertheless, because of the high potential for bioconcentration and absorption to soil matrices, it is likely that PCDFs are persistent in the environment.

5.0 EXPOSURE

5.1 INTRODUCTION

As described in Chapter 2, PCDFs are input to the environment in considerable quantity. As a result, one must suspect the exposure of the human population via food, air, water and dermal vectors. However, as is the case with the PCDDs (NRCC 1981a), data pertinent to estimating exposure to the PCDFs are very inadequate. The data are generally not isomer-specific and are often lacking in precision and accuracy.

In the PCDD monograph (NRCC 1981a), exposure was discussed in two contexts: the exposure route, defined as the activity or use pattern of the contaminated material that leads to an individual being exposed, and the vector, defined as the manner in which the contaminant enters the exposed individual. Since the routes and vectors pertinent to PCDD exposure also apply for the PCDFs, the reader is referred to the background detail in NRCC (1981a); this discussion will focus directly on route and vector combinations for both occupational exposure and exposure of the general population.

5.2 OCCUPATIONAL EXPOSURE

Occupational exposure to PCDFs derives principally from contaminated chemical products either manufactured or used in the workplace. Chemicals considered to be potential sources of PCDFs have been discussed (Chapter 2). Some of the workplaces or occupations in which chemicals contaminated with PCDFs could be encountered include CP manufacturing, shipping, formulation or usage facilities, phenoxy herbicide manufacturing or application and situations where contact may be made with PCBs, e.g. transformer or capacitor maintenance.

5.2.1 CP EXPOSURE

Some of the situations that could result in occupational exposure to the CPs are listed in Table 5-1. Much of the data is from Table 6-7 in NRCC (1981a). Following the style of NRCC (1981a), we made subjective ratings for sources of exposure:

0 Importance not clearly identified
1 Vector possible but no industrial analysis available
2 Vector probably important but no exposure data available
3 Vector has at least minimal qualitative or quantitative support
4 Vector has relatively complete support including exposure/effect analysis.

In some cases, specifically those related to the use of chlorophenols, PCDFs have been detected in the blood and tissue of workers. Data are presented (Table 5-2) (Rappe and Buser 1981) for sawmill workers exposed to 2,3,4,6-tetrachlorophenate and for textile

Table 5-1. Some potential occupational exposure to PCDFs[α] related to CP use (data from NRCC 1981a; Rappe and Buser 1981).

Chemical	PCDF contamination $mg.kg^{-1}$	Identified PCDF homologues	Occupation	Vectors Dermal	Respiratory	Dietary
PCPs[γ]	100-3000	T_4CDFs	Wood Protection/Preservation			
		P_5CDFs (incl.	Chemical handling	2-4	0-4	0-4
		1,2,4,6,8-)	Treatment: Dipping	2	0	0
		H_6CDFs (incl.	Spray	2	0	0
		1,2,3,4,6,8-,	Pressure	4	4	4
		1,2,4,6,8,9-)	Handling treated lumber (incl. planing, trimming, and grading)	4	4	0
		H_7CDFs (incl.				
		1,2,3,4,6,7,8-,				
		1,2,3,4,6,8,9-)	Equipment Maintenance	2	2	0
		O_8CDFs	Commercial yard	2	2	0
			Agriculture			
			Wood waste livestock litter	0	0	0
			Treatment exterior structures	2	2	2
			Interior structures[β]	3	2	0
			Pulp and Paper			
			Slime control[β]	2	2	2
			Wood wastes	0	0	0
			Chemical handling	2	2	2
			Textile Industry			
			Manufacturing	4	3	0
			Preservation of twine, cable, rope, etc.	3	0	0
			Construction Materials			
			Asbestos shingles, concrete blocks, bricks, tiles, etc.	1	0	0
			Treated wood	4	4	0
			Other			
			Leather industry[β]	4	4	4
			Other slime control	2	0	0
			Paint and Stain manufacturing	3	3	0
Phenoxy herbicides	<0.05-0.14[δ]	T_3CDFs T_4CDFs P_5CDFs	Applicators	1	1	0

[α] Vectors sometimes correspond to those known for PCDDs. Specific information is not available for PCDFs in all cases.

[β] Discontinued in Canada 1981 (Agriculture Canada 1980).

[γ] Isomer and homologues may vary with specific formulation.

[δ] May not reflect levels in current formulations.

Table 5-2. Levels of PCDFs associated with blood samples from workers in the textile, tannery and sawmill industries[α] (data after Rappe and Buser 1981).

Person No.	Industry	CPs in urine[δ] µg.mL^{-1}	PCDFs in blood ng.kg^{-1} Octa	Hepta 1,2,3,4,6,7,8-	Hepta 1,2,3,4,6,8,9-	Hexa	Penta	Total
1	Sawmill, loader[β,ζ]	0.04	<3	40	<1	<1	-	
1	Sawmill, loader[γ,ζ]	5.2	<2	22	<1	<1	<1	
2	Sawmill, loader[β,ζ]	0.03	<3	18	<1	<3	-	
2	Sawmill, loader[γ,ζ]	0.83	<2	17	<1	<1	1	
3	Sawmill, packer[β]	<0.05	<3	7	<1	<3	-	
3	Sawmill, packer[γ]	0.11	<2	12	<1	<1	<1	
4	Sawmill, cleaner[β]	<0.02	<3	30	<1	<3	-	
4	Sawmill, cleaner[γ]	0.23	<2	17	<1	<1	5	
5	Sawmill, control[β]	<0.01	<2	3		<1	<1	
6	Textile, mixer[ε]	<0.01	<2	<1		<1	<1	
7	Textile, mixer[ε,ζ]	3.12	10	25	8	<1	<1	
8	Textile, mixer[ε]	0.16	<1	3	<1	<1	1	
9	Textile, mixer[ε]	<0.01	<2	—	<1 —	<1	<1	
10	Textile, mixer[ε,ζ]	0.42	<2	—	<1 —	<1	<1	
11	Tannery[β]	0.55	<3	7	<1	<3	-	
12	Tannery[β,ζ]	0.04	7	18	<1	3	-	
13	Tannery[β,ζ]	0.03	<3	3	<1	<3	-	
14	Tannery[β]	-	<3	3	<1	<3	-	

[α] Sawmill workers were exposed to 2,3,4,6-tetrachlorophenate. Others were exposed to PCP or its derivatives.

[β] Blood sampled 5 months after last exposure.

[γ] Blood sampled 1 month after last exposure.

[δ] Urine sampled 8 months after last exposure.

[ε] Blood sampled 8 months after last exposure.

[ζ] Known exposure to CP solutions.

and leather industry workers following exposure to PCP or its derivatives. Unlike the PCDDs, high levels of O_8CDF do not occur in blood, although in commercial PCPs, O_8CDF can occur at similar levels to those of O_8CDD (e.g. Buser 1975; Table 2-9). Rappe et al. (1983e) reported that the isomers found in blood from textile industry workers otherwise paralleled those found in the CP formulation used. There is no good correlation between PCDF-blood and PCP-urine analysis versus the time lapse since the last exposure. PCP levels in urine would not be expected to be a good indicator of exposure to PCDFs. While the presence of PCP in urine would indicate there had been exposure to PCDF, the absence of PCP in urine would not necessarily mean that there had been no exposure. The analysis of PCDF levels in blood samples is likely a more uniform indicator of exposure. However, even blood levels can be used only as qualitative indicators of exposure since no pharmacokinetic model is available for humans.

Rappe et al. (1983c) point out that they observed a 100-fold variation in both CP levels (CP homologues not specified) in the urine and PCDF levels in the blood between workers in different job categories. There was a reasonable correlation between blood PCDF levels and the number of years some workers had spent manufacturing an unspecified CP formulation (Figure 5-1). The implication is the importance of separating workers by level of exposure for epidemiological purposes.

5.2.1.1 Phenoxy Herbicides

The levels of PCDFs in the 2,4,5-T and 2,4-D formulations now in use are generally unknown. In older formulations the PCDF/PCDD ratio ranges from <0.01 to 4.6 (Table 2-14). 2,3,7,8-T_4CDF is apparently not the dominant T_4CDF homologue in 2,4,5-T formulations. In the absence of data, it must be assumed that PCDFs pose a potential risk to exposed groups.

5.2.2 PCB EXPOSURE

PCDFs are known to be a contaminant of unused commercial PCBs at levels up to 33 $mg.kg^{-1}$ (see Chapter 2). The most toxic isomers are known to be major contaminants. 2,3,7,8-T_4CDF has been identified in North American PCBs at concentrations of up to 2.4 $mg.kg^{-1}$. Other identified isomers include 2,3,6,7-, 2,3,6,8-, 2,3,4,7,8-, 1,2,3,7,8-, 1,2,4,7,8-, 1,2,3,4,7,8-, 1,2,4,6,7,8-, 1,2,4,6,8,9-, 1,2,3,4,6,7,8- and 1,2,3,4,6,8,9-PCDF, (Rappe and Buser 1981, see Chapter 2). Although PCBs have not been manufactured or distributed since 1977, they are still in use in electrical equipment. Thus, human exposure could result from the disposal or repair of such electrical equipment or in buildings with PCB-based heat exchangers, etc., or to bystanders or firefighters when an accident occurs with such equipment (see Section 2.1.1.2).

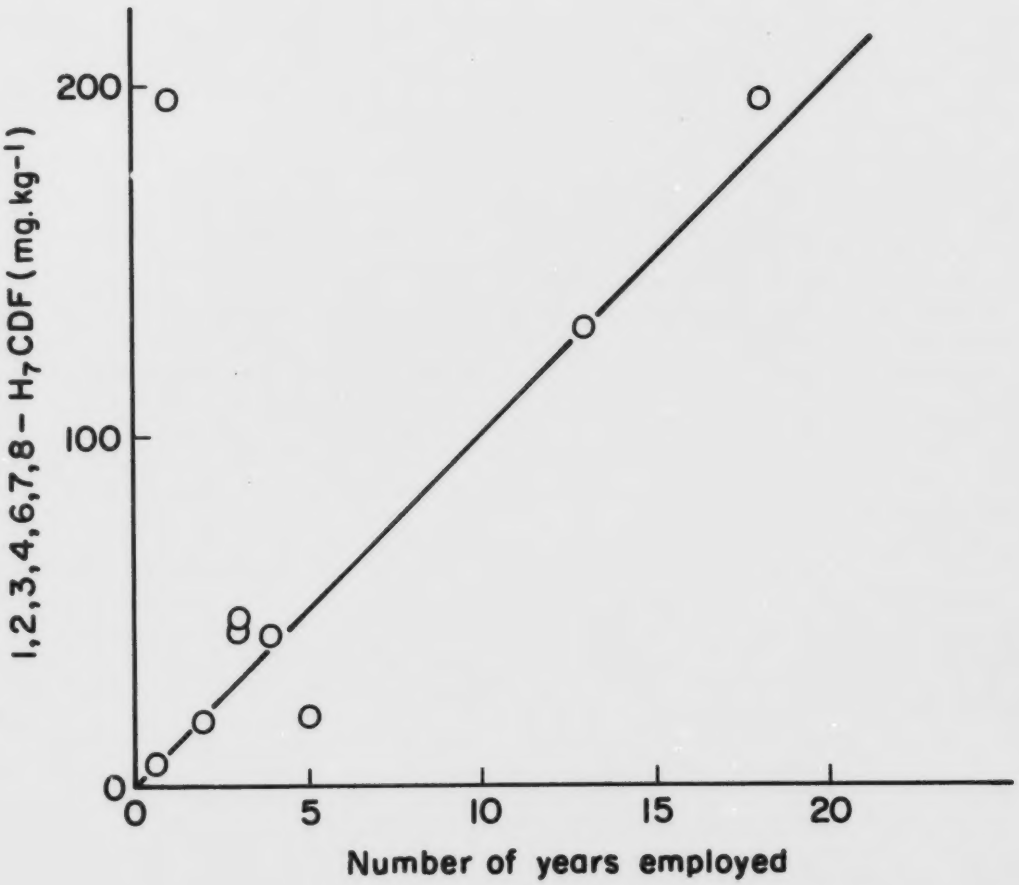


Figure 5-1. Level of 1,2,3,4,6,7,8-H_7CDF in blood plasma versus years of employment during the manufacture of an unspecified chlorophenol (data after Rappe et al. 1983c).

Usually it is necessary to extrapolate from PCB exposure to PCDF exposure. Such extrapolations involve many unvalidated assumptions, e.g. that the volatilities, absorption rates, etc. of the compounds are similar. Additionally, the PCDF contamination levels for the specific situation are often unknown.

There is little information available concerning PCB exposure levels in the electrical industry but such exposure is known to occur in the U.S. (e.g. Fischbein et al. 1982; Moseley et al. 1982; Wolfe et al. 1982).

Moseley et al. (1982) reported that one group of transformer maintenance workers was exposed to an 8-hour time-weighted average (TWA) of 0.01 to 24 $\mu g\text{-PCB}.m^{-3}$. Table 5-3 shows the ambient air PCB levels in the transformer vaults, adjacent areas, and areas some distance away. During the repair operations, 65% of the samples were in excess of the recommended NIOSH exposure limit of a TWA of 1 $\mu g\text{-PCB}.m^{-3}$. The PCB involved contained 13 to 116 $\mu g\text{-}T_4CDF.kg^{-1}$. The highest sample contained 31 $\mu g\text{-}2,3,7,8\text{-}T_4CDF.kg^{-1}$. Based on this, "worst case" ambient T_4CDF levels have been estimated. The authors speculate that the decline in levels following the repair operations is presumably due to the vault ventilation system and the dissipation of the PCBs through, and eventual elimination from, the building by the general air conditioning system.

The report also details levels in personal breathing zone samples categorized by the type of repair (Table 5-4). Concentrations have been normalized to an 8-h TWA but the sample durations ranged from 1 to 6 h. These results indicate that the workers were exposed to up to 2.8×10^{-6} $\mu g\text{-}T_4CDF.m^{-3}$ on an 8-h TWA, about 7.4×10^{-7} $\mu g.m^{-3}$ of this being the 2,3,7,8- isomer.

The report does not discuss dermal or other exposure. It is mandatory in the U.S. (NIOSH 1977) that the electrical maintenance workers wear protective rubber clothing but the efficacy of this rule or of the clothing has not been documented. Inhalation exposure for $2,3,7,8\text{-}T_4CDF$ can be estimated as follows (since the workers are supplied with respiratory masks, this may be an over-estimate):

Assuming a respiration rate of 29 $L.min^{-1}$ (Franklin et al. 1981) and that all the droplets are respirable the inhalation exposure becomes:

$$29 \times \frac{7.4 \times 10^{-7}}{1000} \ \mu g.min^{-1}$$

Correcting for the body weight of a 70 kg man:

$$29 \times \frac{7.4 \times 10^{-7}}{1000 \times 70} = 3.1 \times 10^{-10} \ \mu g.kg\text{-}BW^{-1}.min^{-1}$$

Table 5-3. Ambient air PCB and estimated PCDF levels determined by >10 h air sampling.

Breathing Zone Level	$\mu g.m^{-3}$					
	Pre-maintenance		During-maintenance		4-mon.-post-maintenance	
	PCB	2,3,7,8-T_4CDF[α]	PCB	2,3,7,8-T_4CDF[α]	PCB	2,3,7,8-T_4CDF[α]
Inside Vault	0.1-0.9	3.1-27.9 x 10^{-9}	1.9-55	0.59-17.05 x 10^{-7}	<0.1-1.9	<3.1-58.9 x 10^{-9}
Immediately outside vault	<0.1-0.6	<3.1-18.6 x 10^{-9}	<0.2-2.6	<6.2-80.6 x 10^{-9}	<0.1	<3.1 x 10^{-9}
Generally occupied areas	<0.1-0.2	<3.1-6.2 x 10^{-9}	0.3-1.4	9.3-43.4 x 10^{-9}	<0.1-0.2	<3.1-6.2 x 10^{-9}

[α] Estimated from the 31 $\mu g.kg^{-1}$ detected in one PCB sample and assuming similar volatility, absorption rates, etc.

Table 5-4. PCB and estimated 2,3,7,8-T_4CDF concentrations in personal breathing zone samples (after Moseley et al. 1982).

Source	8-h TWA $\mu g.m^{-3}$	
	PCB	2,3,7,8-T_4CDF[α]
Oil drain-plug repair and cleanup	16.7-24.0	$5.2\text{-}7.4 \times 10^{-7}$
Sight gauge and oil drain-plug repair and cleanup	3.2-7.0	$0.99\text{-}2.2 \times 10^{-7}$
Cleanup of PCB leak only	0.01-0.4	$0.31\text{-}12.4 \times 10^{-9}$
Secondary oil leak repair and cleanup	0.7-12.4	$0.22\text{-}3.84 \times 10^{-7}$

[α] Estimated assuming similar volatility, absorption rates, etc.

Thus, the theoretical inhalation exposure to 2,3,7,8-T_4CDF over an 8-h working day for a 5-d week would be:

$$3.1 \times 10^{-10} \times 60 \times 8 \times 5 = 7.2 \times 10^{-7} \text{ µg.kg-BW}^{-1}\text{.wk}^{-1}$$

Dermal exposure cannot be estimated from the air concentration and may be considerable due to touching capacitor parts.

Wolfe et al. (1982) and Fischbein et al. (1982) found that plasma and adipose PCB concentrations among persons employed in the U.S. in capacitor manufacturing correlated with the estimated relative degree of exposure to PCB and with the duration of the exposure. A high prevalence (37%) of dermatological abnormalities was found among such workers. Mean air PCB levels ranged up to 11 mg of Aroclor 1016 per m^3. Actual PCDF exposure was unknown.

Digernes and Astrop (1982) report PCB concentrations of 56-81 $ng.m^{-3}$ in air close to video display terminals in an office in Norway. This was 50-80 times the level outside the building and there were qualitative differences in the Aroclors detectable inside and outside the building. Skin rashes had been reported in the video display terminal users. MacLeod (1981) reports that in the U.S., high PCB concentrations were detected in kitchens (0.15-0.58 $µg.m^{-3}$), offices (0.8-0.11 $µg.m^{-3}$) and laboratories (0.2-0.4 $µg.m^{-3}$). Outdoor concentrations in the U.S. generally ranged from 0.004 to 0.02 $µg.m^{-3}$. MacLeod (1981) speculates that fluorescent light installations or other appliances could be a source. Czerkawski (1983) estimates that over 60 million PCB-containing fluorescent light units (60% of the total units in use) and additional high density discharge light units are in use in Canada. The total PCB in these units is in the order of 2.5 to 3 x 10^6 kg. This could represent 15 kg of PCDF (see Section 2.3.1). In a room in which a fluorescent light ballast had burned out, the PCB levels were 11.6 $µg.m^{-3}$ and remained high for several months. Levels in kitchens were generally higher than in other residential rooms, and just as high as the industrial and academic labortories tested.

5.2.3 EXPOSURE OF THE GENERAL POPULATION

There are few data on exposure of the general population. Potential routes for exposure of the general population to PCDFs include contaminated food, air and industrial products (Table 5-5). Additionally, exposure could result from accidents like those discussed elsewhere in this document (e.g. Seveso, Yusho, etc.).

The toxicological significance or bioavailability of PCDD and PCDF levels associated with incinerators is debatable. Helder et al. (1982) reported that exposure to large amounts of fly ash (300 g per tank contaminated with about 4 $mg.kg^{-1}$ of PCDDs and PCDFs; see Table 2-16) did not impair growth, health, development or behavior of rainbow trout yolk-sac fry. However, the addition of about 0.5 g per tank of an

Table 5-5. Routes of exposure of the general population to PCDFs.

Source	Vectors[ε]		
	Dermal	Respiratory	Dietary
Food[δ] - Fish	0	0	4
- Shellfish	0	0	4
- Dairy	0	0	4
- Poultry	0	0	4
Incineration[δ]	0	2	0
CP treated wood	2	2	2
Other CP products			
- Leather[α]	1	1	0
- Herbicides	1	1	0
- Paints	1	0	0
- Stains[α]	1	1	0
- Rubber			
- Textiles[β]	1	0	0
- Fuels[γ]	0	4	4
- Disinfectants			
Electrical PCB use[α] (includes transformers, capacitors, fluorescent light tubes, etc.)	4	4	0
Other PCB use[α]			
e.g. - copy paper	1	1	0
- fire retardants	1	1	0
- inks	1	1	0
- paints	1	1	0
- adhesives, sealants	1	1	0
- cutting and immersion oils	1	0	0

[α] Use of PCBs in these products has been prohibited since September 1977, but many products still in use in Canada.

[β] Use now limited (Agriculture Canada 1980).

[γ] Includes auto, fireplaces, cigarettes.

[δ] Primary sources include PCBs, CPs, etc.

[ε] Vector rating as in Table 5-1. Much of the data is from Table 6-10 in NRCC (1981a).

equivalent "cleaned up" fly ash (chromatography on acid-, base- and silver nitrate-modified silica gel then on a basic aluminium oxide column) was fatal for 100% of the fry. Van den Berg et al. (1983) reported that rats fed fly ash and a fly-ash extract in their diet absorbed the extract more efficiently. These data suggest that the soot-matrix reduces the bioavailability of the PCDDs and PCDFs.

Inhalation could be a significant exposure route for incinerator effluents. There is a paucity of appropriate inhalation studies with laboratory animals. Such studies would be useful. It might also be useful to monitor bioavailability exposure markers such as levels in adipose tissue from cattle grazing near an incinerator or from fish in adjacent waters. Olie et al. (1983) concluded that regular consumption of food grown in the vicinity of an incinerator could be an exposure route. McLaughlin and Pearson (1984) found no evidence that PCDDs or PCDFs were accumulating in soil adjacent to a municipal incinerator in Ontario (see Section 2.4.2).

Ryan and Williams (1983) reported that human adipose tissues from residents of Canadian Great Lakes areas have detectable amounts of PCDDs and PCDFs. Four of five samples analyzed were positive for 2,3,7,8-T_4CDF (average 2.8 $\mu g.kg^{-1}$) and six of six samples analyzed were positive for 2,3,4,7,8-P_5CDF (average-- 17 $\mu g.kg^{-1}$; range-- 2 to 60 $\mu g.kg^{-1}$).

6.0 METABOLIC DISPOSITION

6.1 ABSORPTION AND TISSUE DISTRIBUTION

In NRCC (1981a) it was pointed out that the rate of absorption of 2,3,7,8-T_4CDD was highly dependent on the matrix and on the route of administration. Different PCDD congeners were also found to accumulate differentially in various organs and species (NRCC 1981a).

Absorption data are available for oral and i.v. dosing of 2,3,7,8-T_4CDF in several species. Birnbaum et al. (1980, 1981) and Decad et al. (1981a, b; 1982) studied the fate of [^{14}C]2,3,7,8-T_4CDF in male Fisher rats, male Rhesus monkeys, male guinea pigs and male C57BL/6J and DBA/2J mice. Their data on excretion and clearance are discussed in Section 6.2. The rats were dosed by gavage (30.6 and 306 $\mu g.kg\text{-}BW^{-1}$) or i.v. (30.6 $\mu g.kg\text{-}BW^{-1}$), the monkeys were dosed i.v. (30.7 $\mu g.kg\text{-}BW^{-1}$), the guinea pigs were dosed i.v. (6 $\mu g.kg\text{-}BW^{-1}$) or by gavage (6.1 $\mu g.kg\text{-}BW^{-1}$) and both strains of mice were dosed i.v. (30.6 $\mu g.kg\text{-}BW^{-1}$).

In rats and guinea pigs 2,3,7,8-T_4CDF was readily absorbed from the gastrointestinal tract. The extent of absorption was not a function of the doses administered (Birnbaum et al. 1980) and the distribution patterns were not dependent on the route of administration. About 90% of the gavage dose was initially absorbed from the gut in rats and guinea pigs, 90% in C57BL/6J and 70% in DBA/2J mice. In all species but the DBA/2J mice, major transfer was initially to the liver and in lesser amounts to muscle, followed by some redistribution to skin and adipose tissues. More than 95% of the tissue burden in rats and guinea pigs appeared to be unmetabolized 2,3,7,8-T_4CDF. After three days, the distribution of radicactivity was similar in animals dosed by gavage and intravenously. The initial distribution patterns in mice were similar but after 30 days more 2,3,7,8-T_4CDF was retained in the adipose tissue of DBA/2J mice than in that of C57BL/6J mice, possibly because adipose tissue represented a higher percentage of DBA/2J mice body weight. The tissue distribution patterns after 3 to 21 days for rat, monkey, mouse and guinea pig are summarized in Table 6-1. While the general patterns were similar, differences in the relative distribution of 2,3,7,8-T_4CDF between liver and fat tissue are apparent in some species, possibly reflecting the percentage of the body that is adipose tissue.

When a mixture of PCDFs (two T_4CDFs, four P_5CDFs, four H_6CDFs) was administered i.p. to mice, the PCDFs were found mainly in liver, spleen and fat tissue with lower concentrations in kidney, testes and lung (Morita and Oishi 1977). In all except the liver tissue, the GLC pattern after three days was similar to that of the administered mixture. Several peaks were diminished in the liver chromatogram. After four weeks, while most tissues still showed the same GLC pattern, the pattern for liver tissue had changed dramatically. This suggests

Table 6-1. Tissue distribution of radioactivity after i.v. administration of [^{14}C]2,3,7,8-T_4CDF in rats, monkeys, mice and guinea pigs (after Birnbaum et al. 1980, 1981; Decad et al. 1981a, b).

	% recovered dose[α]				
	Rat Day 3	Monkey Day 21	Mouse Day 3		Guinea pig Day 3
Tissue			C57BL/6J	DBA/2J	
Liver	7.0	1.7	25.5	19.3	23.3
Fat	13.8	6.2	3.2	25.7	45.2
Skin	1.4	4.0	3.4	3.8	13.6
Muscle	0.5	2.7	1.7	6.2	12.4
Excreted					
Feces	74.8	72.0	57.8	31.7	3.7
Urine	2.4	13.2	8.4	13.2	1.8

[α] Values have been normalized to account for the different recoveries.

that there is significant metabolism only in liver tissue where different homologues are metabolized at different rates (see Section 6.2). The chronic homologue pattern could change if slow metabolism occurs in other tissues.

Many of the earlier studies on the tissue distribution of PCDFs used human tissue from patients who died from Yusho disease[α]. While autopsy samples are usually preserved after dissection, there could be some breakdown of PCDFs during storage.

Japanese Yusho oil has been reported to contain mainly tetra, (~22%), penta (~46%) and hexa (~30%) PCDFs with smaller amounts of tri PCDFs (~2%) (e.g. Morita et al. 1977; Buser et al. 1978c). At least 40 PCDF congeners (including 2,3,7,8-T_4CDF and 2,3,4,7,8-P_5CDF as major components) have been reported (Table 6-2) (Buser et al. 1978c; Rappe et al. 1979b). Nagayama et al. (1977) reported that PCDFs occurred in similar concentrations in adipose and liver tissue of Yusho patients, in contrast to the PCBs ingested at the same time, which occurred mainly in adipose tissue. They reported that the persistent congeners were almost exclusively penta and hexa homologues.

Kuroki and Masuda (1978) found that some PCDF isomers persisted in the liver and adipose tissue of Yusho patients for up to nine years after the exposure. Penta homologues (1,2,4,7,8- and 2,3,4,7,8-) were present in higher concentrations than 2,3,6,8- and 2,3,7,8-T_4CDF, although the latter were reported in similar or higher relative concentration in the oil ingested (Table 6-2). They found isomer concentrations to be generally 2- to 8-fold higher in adipose tissue than in liver tissue. While the absolute tissue concentrations differed between patients, there was some correspondence between tissue concentrations and the time period between exposure and death.

More recently, Rappe et al. (1979b) identified ten PCDF homologues in liver tissue taken from Japanese Yusho patients about 18 months after the exposure; in comparison, at least 40 homologues are present in Yusho oil. They identified and quantified the isomers in the samples and found structural similarities in the groups of isomers retained and in those cleared by the patients (Figure 6-1). All isomers with two vicinal hydrogenated C-atoms in at least one of the two rings were cleared; most of the retained isomers had chlorine in all the lateral positions. Masuda and Kuroki (1982) analyzed liver and adipose tissue from Yusho patients for specific PCDF isomers (Table 6-3). They also found that all the retained isomers had 3 or 4 Cl atoms in lateral ring positions and no vicinal hydrogens.

[α] Yusho disease resulted from ingestion of PCB-contaminated rice oil in two major incidents in Japan and Taiwan in 1968 and 1979, respectively (see Chapter 7).

Table 6-2. PCDFs identified in Japanese Yusho oil (from Rappe et al. 1979b).

Homologue		Relative Concentration[α]
Tetra:	2,4,6,8(+1,3,6,7)[β]	Trace
	2,3,6,8	2
	2,3,7,8	3
	2,3,6,7	Trace
	3,4,6,7	1
	4 unknown	
Penta:	1,2,4,6,8	1
	1,3,4,7,8	1
	1,2,4,7,8	2
	(1,3,4,7,9)	Trace
	1,2,3,7,8	2
	2,3,4,6,8	1
	1,2,3,4,8	2
	1,2,3,6,7	Trace
	1,2,6,7,8	2
	2,3,4,7,8(+1,3,4,8,9)	3
	2,3,4,6,7	2
	1,2,4,8,9	Trace
	2 unknown	
Hexa:	1,2,3,4,6,8	1
	1,3,4,6,7,8	1
	1,2,4,6,7,8	1
	1,2,4,6,8,9	1
	1,2,3,4,7,8	2
	1,2,3,4,6,7	2
	1,2,3,6,7,8	1
	1,2,3,6,8,9	ND[γ]
	2,3,4,6,7,8	1
Hepta:	1,2,3,4,6,7,8	1
	1,2,3,4,6,7,9	ND[γ]
	1,2,3,4,6,8,9	ND[γ]
	1,2,3,4,7,8,9	ND[γ]

[α] Relative concentrations: 1 (low), 2 (medium), 3 (high).

[β] Homologues in parentheses only minor constituents, if present at all.

[γ] Not detected.

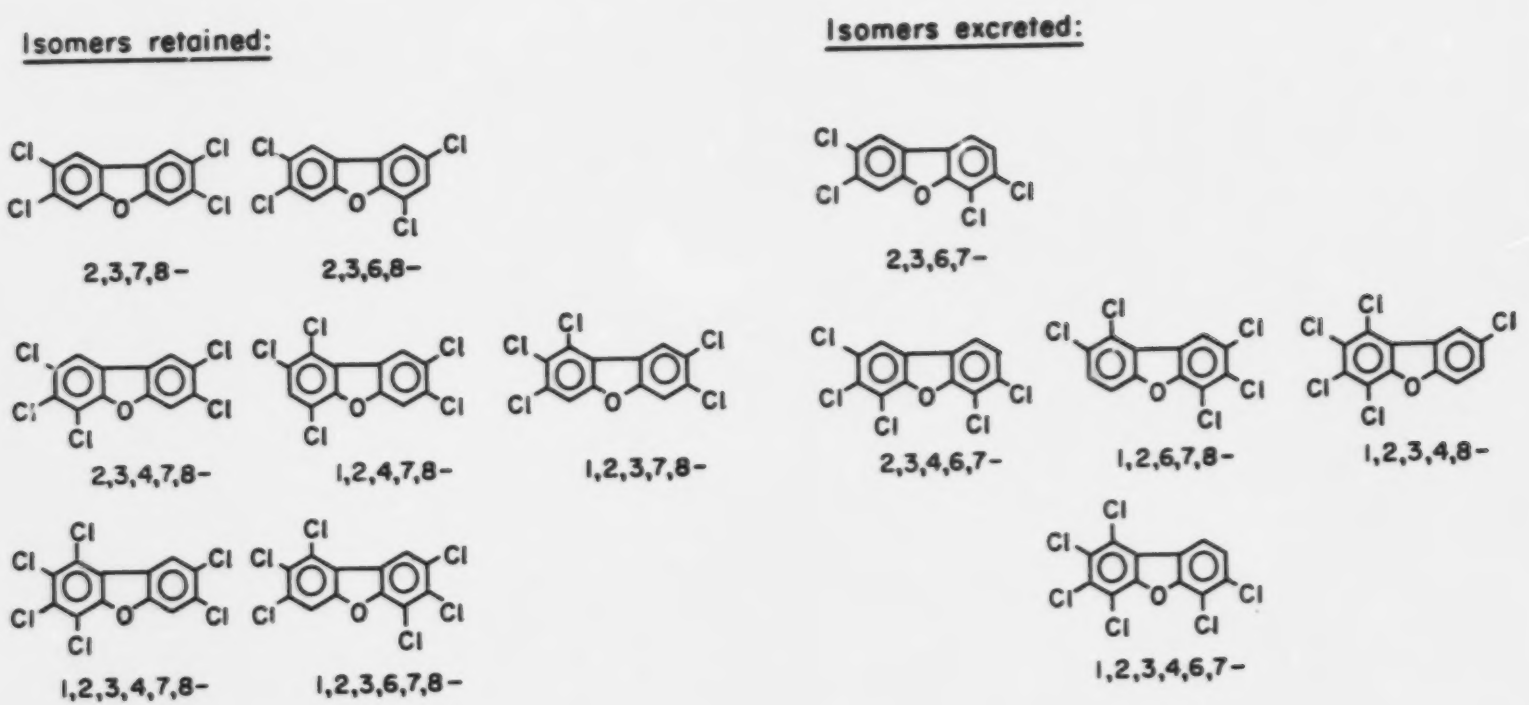


Figure 6-1. PCDF isomers retained and excreted from the liver of Japanese Yusho patients (from Rappe et al. 1979b).

Table 6-3. PCDF isomers retained in the liver and adipose tissue of Japanese Yusho patients, expressed as percentage of total PCDF retained (after Masuda and Kuroki 1982).

Years from exposure to death	Tissue	Isomer as % of total				
		2,3,6,8-	2,3,7,8-	1,2,4,7,8-	2,3,4,7,8-	1,2,3,4,7,8- 1,2,3,6,7,8-
1	liver	4	1.7	40.3	39.2	14.8
1	liver	4	1	20	60	15
	adipose	6.5	3.2	10.8	61.3	19
4	liver	6.7	<2	20	66.7	6.7
	adipose	6.2	<0.3	15.6	62.5	15.6
7	adipose	11.1	22.2	44.4	5.5	27.8
9	liver	<3.1	<3.1	12.5	62.5	25
	adipose	<0.7	<0.7	28.6	71.4	<0.7

In each of a set of four blood samples, two collected in Japan 11 years after exposure and the others collected in Taiwan one year after exposure, 2,3,4,7,8-P_5CDF was predominant (Rappe et al. 1983c).

Rappe et al. (1983e) analyzed various organs from a Taiwanese Yusho baby for PCBs and PCDF isomers (Table 6-4). The Taiwanese oil has not been analyzed for isomers, so it is unclear how this pattern compares with the isomers ingested. While PCBs were found in several tissues at levels which seemed to correlate with tissue fat levels, PCDFs were found only in adipose and liver.

Rappe et al. (1983e) analyzed the PCDF isomer content of liver and kidney from a person who died 3-4 years after being occupationally exposed to phenoxy acid herbicides (including 2,4-D and 2-methyl-4-chlorophenoxy acid (MCPA)) and to 2,4,6-T_3CP during their production in Sweden. They found the highest levels in the liver (total ≈ 165 ng.kg-tissue^{-1}) with 2,3,4,7,8-P_5CDF, 1,2,3,4,6,7,8-H_7CDF and H_6CDFs dominant in both types of tissue. T_4CDFs and O_8CDFs were not detectable at detection limits of 1 and 3 ng.kg^{-1}, respectively. The isomer pattern to which the man was exposed is unknown.

Kuroki et al. (1980) administered a mixture of PCDFs orally to monkeys for about thirty days, and as a single dose i.p. to rats. The administered mixture consisted of 1,2,7,8-, 2,3,7,8-, 1,2,3,7,8-, and hexa PCDF homologues in known proportions. Monkey liver and adipose tissue were removed at the end of the dosing period and rat liver was removed 5 d after the dose. The accumulative properties of the homologues differed. 2,3,4,7,8-P_5CDF was most highly accumulated in the liver, when expressed as a percentage of the intake retained. A large difference in the extent of accumulation of 2,3,4,7,8-P_5CDF in monkeys and rats (0.8 to 5.2% versus 76 to 82% respectively) was attributed by the authors to a species difference rather than to the different routes of administration. When the data were expressed as the ratio of the isomer fraction of the total PCDF in a tissue to the isomer fraction in the mixture administered, there was also some evidence of a difference, which could be attributed either to the different species or to the different administrative routes (Table 6-5).

Yoshihara et al. (1981) administered 13 individual PCDF congeners (2,8-, 1,2,7,8-, 1,3,6,7-, 1,3,6,8-, 2,3,6,7-, 2,3,7,8-, 1,2,3,7,8-, 1,2,4,6,8-, 1,2,6,7,8-, 2,3,4,6,7-, 2,3,4,7,8-, 1,2,3,4,6,7-, 1,2,3,4,7,8-) by i.p. injection (1, 5 or 10 mg.kg-BW^{-1}, single dose) to young male Wistar rats. They reported the percentage of the administered dose of each congener retained in the liver after 5 days (Table 6-6). Among the 7 PCDFs having vicinal hydrogens, less than 1% of the dose was retained for 6 congeners and about 7% was retained for the other. However, 3 congeners with only isolated hydrogens were also retained at very low levels. 2,3,7,8-T_4CDF and 2,3,4,7,8-P_5CDF, similar in their structure, MFO inducibility and toxicity, were retained to very different extents. These results support the observations

Table 6-4. Levels of PCDFs and PCBs in tissue from a Taiwan Yusho baby (after Rappe et al. 1983e).

Isomer	Concentration ng.kg-tissue^{-1}				
	Adipose	Liver	Muscle	Omentum	Diaphragm
2,3,7,8-T_4CDF	17	60	ND	ND	ND
1,2,4,7,8-P_5CDF	14	42	ND	ND	ND
1,2,3,7,8-P_5CDF	44	194	ND	ND	ND
2,3,4,7,8-P_5CDF	68	91	ND	ND	ND
1,2,3,4,7,8-H_6CDF	88	193	ND	ND	ND
PCBs	316	27	38	64	46

Table 6-5. Distribution of PCDF isomers in monkey and rat tissue relative to the isomers in the mixture administered (after Kuroki et al. 1980).

	% isomer of total PCDF in organ / % isomer of total PCDF administered		
	Monkeys[α]		Rat[β]
Isomer	Adipose	Liver	Liver
1,2,7,8-T_4CDF	<0.1	$<1.7 \times 10^{-2}$[γ]	$<5.4 \times 10^{-3}$
2,3,7,8-T_4CDF	<0.9	0.2 - 0.6	0.01 - 0.2
1,2,4,7,8-P_5CDF	<1.5	2.4 - 3.7	$<7.5 \times 10^{-2}$
1,2,3,7,8-P_5CDF	1.6 - 1.2	1 - 1.3	1.4 - 1.6
2,3,4,7,8-P_5CDF	22 - 60.8	11.8 - 35.2	6 - 8.2
H_6CDF	<1.0	0.9 - 5.6	10.9 - 15.8

α 2 monkeys (1M, 1F: 4-6kg) fed 1.25 - 2.5 μg.kg-diet^{-1} for 29-32 d.

β 4 rats (M: 100g) given i.p. 10 mg.kg-BW^{-1} single dose.

γ "<" indicates tissue levels below detection limits (0.01 mg.kg^{-1}).

Table 6-6. Retention of PCDF congeners in rat liver 5 days after a single i.p. injection (after Yoshihara et al. 1981).

Congener	Dose mg.kg-BW^{-1}	Retained[α] %	Experiment
2,3,4,7,8	1	69.5±19.7	1
	1	65.2±12.4	2
	1	96.5±12.0	3
2,3,7,8	1	3.8±0.5	1
1,2,3,7,8	10	44.0±4.4	1
1,2,3,4,7,8	10	19.5±3.7	1
1,2,7,8	1	ND[β]	2
2,3,6,7	1	ND[β]	2
1,2,6,7,8	1	0.12±0.06	2
2,3,4,6,7	1	7.4±1.0	2
1,2,3,4,6,7	1	0.59±0.39	2
2,8	10	ND[β]	3
1,3,6,7	5	<0.02	3
1,3,6,8	5	<0.15	3
1,2,4,6,8	5	0.21±0.2	3

[α] Values represent mean ± SD of four rats.

[β] Concentration in liver less than 0.001 μg.g^{-1}.

(above) of Rappe et al. (1979b) and indicate that hepatic distribution does not generally appear to be directly associated with acute toxicity.

6.1.1 PLACENTAL TRANSFER AND TRANSFER BY NURSING

Nagayama et al. (1980) investigated the transfer of PCDFs to the fetuses and offspring of mice. Dams were fed a diet containing 0.6 $\mu g.kg^{-1}$ of a mixture of PCDFs for either 18 d after mating or 14 d after delivery. The dams, fetuses and offspring were analyzed for PCDFs. Again, different homologues accumulated to different extents and cleared from the dam tissue at different rates after dosing ceased. One penta isomer (not identified, peak "e" in Table 6-7), which was originally present in the highest concentration, cleared almost completely from the liver after two weeks.

PCDFs were reported to be transferred in relatively small amounts across the placenta (0.003% of the total maternal ingestion was found in the fetuses) and in larger amounts to the offspring via milk (0.07% of the maternal ingestion had been transferred after 1 week and 0.14% after two weeks). This transfer is similar to that previously reported for PCBs (Masuda et al. 1978; 1979). When the data are expressed as the ratio of the isomer fraction of the total PCDF in a tissue to the isomer fraction in the mixture administered (Table 6-7), it is seen that certain homologues are preferentially excreted from the dams, some being transferred to the offspring, while others are retained in their liver.

6.2 CLEARANCE AND EXCRETION

Matthews and Kato (1979) speculated that PCDFs may be polar enough to be excreted in the bile prior to metabolic alteration. There is some evidence that PCDFs are excreted rapidly relative to PCBs, which are less polar and generally not excreted at an appreciable rate prior to metabolism (e.g. Norstrom et al. (1976)). Matthews and Kato (1979) also speculated that much of the excreted PCDFs would be reabsorbed in the intestine and returned to the liver. Therefore, they predicted that the rate of elimination would be largely dependent on the rate of metabolism in the liver.

Different species clear PCDFs from their bodies at different rates. The rate also differs for different homologues and depends on the route of administration. Whole-body and tissue half-lives for 2,3,7,8-T_4CDF administered i.v. to mice, rats and monkeys are summarized in Table 6-8.

Morita and Oishi (1977) administered 0.5 mg of a mixture of PCDFs (average 4.7 Cls) i.p. to male mice. They described the

Table 6-7. Distribution of PCDF isomers in mouse tissue relative to distribution in mixture administered.

		% isomer of total PCDF in tissue / % isomer of mixture PCDF administered						
		Mice killed on day 18 of pregnancy			Mice killed 14 d after delivery			
Peak	Isomer	Whole body	Liver	Fetuses	Whole body	Liver	1-w old offspring	2-w old offspring
a	tetra	$<6 \times 10^{-2}$	1.1	<1	<1.4	$<9 \times 10^{-4}$	<0.19	<0.17
b	tetra	$<1 \times 10^{-2}$	$<5.6 \times 10^{-5}$	<0.17	<0.25	$<1.6 \times 10^{-4}$	$<3.3 \times 10^{-2}$	$<3.1 \times 10^{-2}$
c	2,3,7,8	1.7	1.1	2.1	>0.18, <1.8	2.1×10^{-2}	0.74	$<2.2 \times 10^{-2}$, 0.22
d	penta	0.68	1.1	<0.5	>0.73, <7.3	2.5	1.4	1.8
e	penta	1.1	0.76	1.2	<0.16	5.7×10^{-2}	0.43	$>1.9 \times 10^{-2}$, <0.19
f	2,3,4,7,8	1.4	2.8	<0.2	6.3	6.2	3.9	6.2
g	hexa	>0.06, <0.6	2.1	<1	>1.4, <14	3.9	3.0	5.1

Table 6-8. Estimated clearance rates (expressed as half-life ($t_{\frac{1}{2}}$)) for 2,3,7,8-T_4CDF from mammalian tissues after i.v. dosing.

Species	Half life (d)						Note	Reference
	Whole body	Liver	Fat	Muscle	Skin	Blood		
Male Fisher rat	<2	0.1 1.25	3.75	0.02 0.72	0.45 11.08	0.02 1.14	Compartment 1 Compartment 2	Birnbaum et al. (1980)
Male Rhesus monkey	~8					0.001 0.04	Compartment 1 Compartment 2	Birnbaum et al. (1981)
Male (C57BL/6J) mice	2	 1.9	 1.6	0.001 1.1	0.002 4.0		Compartment 1 Compartment 2	Decad et al. (1981b)
Male (DBA/2J) mice	4	 1.8	 7.0	0.002 4.0			Compartment 1 Compartment 2	Decad et al. (1981b)
Male guinea pigs	20	0.16 ?		0.65 ?		[α]	Compartment 1 Compartment 2	Decad et al. (1981a)

[α] 2,3,7,8-T_4CDF was rapidly cleared from blood into tissues.

distribution of the PCDFs in various tissues (see Section 6.1). When the GLC patterns of PCDFs in liver tissues were examined at intervals from 1 day to 8 weeks after administration, it was apparent that different homologues were metabolized or cleared at different rates. The peak tentatively assigned as 2,3,7,8-T_4CDF cleared relatively rapidly with a half-life of about 1 week in mouse liver. The GLC pattern of other tissues, including fat, changed little during the 8 weeks, suggesting that PCDFs once deposited are relatively persistent in fat tissue. The estimated overall half-life for the mixture administered i.p. to mice was about two weeks.

As discussed in Section 6-1, Birnbaum et al. (1980, 1981) and Decad et al. (1981a, b) studied the fate of 2,3,7,8-T_4CDF in rats, monkeys, guinea pigs, and mice. The dosing regimes are summarized in Section 6.1.

In the rat dosed i.v. or by gavage, 2,3,7,8-T_4CDF metabolites were readily excreted in the feces; more than half was excreted within two days and none of this radioactivity cochromatographed with the parent compounds (Birnbaum et al. 1980). Less than 6% of the radioactivity was recovered in the urine, most of this was excreted on day 1. The clearance rate was different for each type of tissue (Table 6-8) and there was a 2-compartment exponential decay from all tissues except fat.

In male Rhesus monkeys dosed i.v., less than 10% of the total dose remained in the body after three weeks. About four times as much 2,3,7,8-T_4CDF was excreted in the feces as in the urine. The excreted radioactivity was mainly in the form of metabolites. Clearance from monkeys was slower than from rats (Table 6-7).

Guinea pigs dosed i.v. appeared to excrete 2,3,7,8-T_4CDF to equal extents in feces and in urine, but less than 9% of the total dose was eliminated by each route during the nine days of the experiment. While more than 90% of the radioactivity extracted from the urine was in the form of one or more metabolites, radioactivity extracted from the feces cochromatographed with 2,3,7,8-T_4CDF. The extreme sensitivity of guinea pigs to the toxic effects of 2,3,7,8-T_4CDF (see Chapter 7) may be attributed to the slow clearance and metabolism. The similarities between 2,3,7,8-T_4CDF and 2,3,7,8-T_4CDD disposition in guinea pigs are striking (NRCC 1981a) and indicate that the chemicals could have similar modes of action, although the proportionate routes of excretion differ (NRCC 1981a).

In both strains of mice dosed i.v., much of the initial 2,3,7,8-T_4CDF dose was excreted within 10 days as metabolites in the feces (80% for C57BL/6J and 55% for DBA/2J). The whole body half-lives of 2 and 4 d for C57BL/6J and DBA/2J respectively, and the greater retention in DBA/2J fat may simply reflect the greater adipose content of this strain.

Rappe et al. (1983c) analyzed blood samples taken from Taiwanese Yusho patients one and two years after exposure. They found the reduction in 2,3,4,7,8-P_5CDF in the year between sampling periods to be only 20% and in 1,2,3,4,7,8-H_6CDF to be 15%. This suggests that these isomers have a half-life in humans of more than one year. As mentioned in Section 6.1, 2,3,4,7,8-P_5CDF was still detectable in blood taken from Japanese Yusho patients 11 years after exposure.

6.3 CATABOLISM

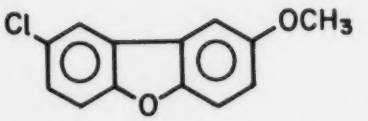
As described in the previous sections, when 2,3,7,8-T_4CDF was fed to various mammals, the excreted radioactivity did not generally co-chromatograph with the parent material; in most cases, one or more presumed metabolites could be detected but no attempt was made to identify them.

Veerkamp et al. (1981) investigated the metabolism of some PCDFs by rats. Male albino Wistar rats were given single oral doses (250 mg.kg-BW^{-1}) of 2-M_1CDF, 2,8-D_2CDF, 2,3,8-T_3CDF or O_8CDF suspended in peanut oil. Feces and urine were collected and fat and liver were analyzed when the rats were killed after one week. Differentiation between hydroxy and methoxy metabolites was possible only in the acidified fat extract, because of the methylation step in the method; thus only two of the $C_{12}H_5OCl_2(OCH_3)$ metabolites from 2,8-D_2CDF can definitely be identified as hydroxy metabolites. The mono-, di- and tri-CDFs yielded mono- and dihydroxy or methoxy derivatives, but metabolites containing sulphur were detected only with the mono- and di-CDFs (Table 6-9). The authors point out that since the analytical method involved hydrolysis and methylation, these structures may be formed during the work-up procedure from glutathione or mercapturic-acid conjugates. No metabolites of O_8CDF were detected. The structure of a number of metabolites was assigned by comparing the mass spectrum with those of authentic materials (Figure 6-2).

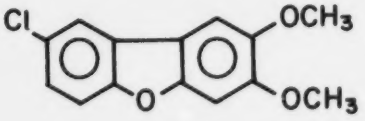
In contrast to the PCDDs, where hydroxy derivatives with a substitution only at the 2,3 positions have been isolated, PCDF metabolites apparently show a wider substitution pattern; five mono-methoxy derivatives were found for 2,8-D_2CDF.

Table 6-9. PCDF metabolites in rats (after Veercamp et al. 1981).

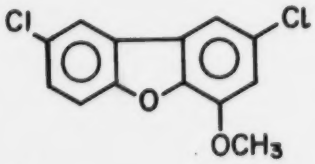
Parent	Molecular Formula of Metabolite	No. Isomers
2-M_1CDF	$C_{12}H_6OCl(CH_3)$	3
	$C_{12}H_6OCl(SH_3)$	3
	$C_{12}H_6OCl(OCH_3)_2$	1
2,8-D_2CDF	$C_{12}H_6OCl(OCH_3)$	1
	$C_{12}H_6OCl(OCH_3)_2$	1
	$C_{12}H_5OCl_2(OCH_3)$	5
	$C_{12}H_5OCl_2(SCH_3)$	1
	$C_{12}H_4OCl_2(OCH_3)_2$	4
2,3,8-T_3CDF	$C_{12}H_4OCl_3(OCH_3)$	5
O_8CDF	No metabolites detected	



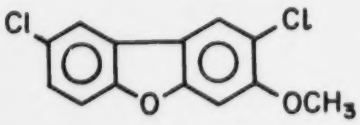
- Formed from 2-M_1CDF and 2,8-D_2CDF
- Found only in urinary extract



- Formed from 2,8-D_2CDF
- Found only in urinary extract



- Formed from 2,8-D_2CDF
- Found in urinary and fecal extracts



- Formed from 2,8-D_2CDF
- Found in urinary and fecal extracts

Figure 6-2. Possible PCDF metabolites in rats (data after Veercamp et al. 1981).

7.0 TOXICOLOGY

7.1 THE SELECTION OF CRITICAL CRITERIA

Assessment of the toxicological effects for a group of compounds such as the PCDFs requires a wide background knowledge. Since the mechanisms of toxicity of the PCDFs are thought to be similar to those of the PCDDs, the reader will find the discussion in NRCC (1981a) useful. Less research has been directed to the PCDFs than to PCDDs and the general characteristics of the effects of PCDFs on biological systems are not as well characterized. The effects selected as critical to the toxicologic hazard assessment of PCDFs have therefore been based on the criteria selected for PCDDs (NRCC 1981a). These effects include:

- Acute lethality
- Subacute toxicity (including mutagenicity and effects on the immune system)
- Reproductive effects (including teratogenicity)
- Chronic toxicity (including carcinogenicity)

The generalities pertinent to each of the above effects have been discussed in NRCC (1981a), as well as the difficulties encountered in trying to extrapolate toxicity data between animal species or between exposure levels. The data presented here pertain to a number of different animal species and strains. It must be remembered that the sensitivity and toxicologic response to a chemical can differ considerably between species.

7.2 LABORATORY ANIMAL TOXICITY

7.2.1 GENERAL SIGNS AND SYMPTOMS

The signs and symptoms associated with mammalian exposure to toxic doses of the PCDFs are similar to those associated with exposure to the PCDDs (NRCC 1981a). The ultimate cause of death in most animals exposed to PCDFs or PCDDs is unknown; apparently, monkeys exposed to 2,3,7,8-T_4CDD die of pancytopenia (Allen *et al.* 1977). Exposure to toxic levels of PCDD or PCDF generally results in an immediate, dose-related and progressive (until death) loss of body weight which is not related to food intake (Moore *et al.* 1979).

External signs of PCDF toxicity differ somewhat between mammalian species. Monkeys receiving single oral (gavage) doses of 500 to 1500 μg-2,3,7,8-T_4CDF.kg-BW^{-1} showed dose-related facial edema, dry skin, thickening of the skin, enlargement of hair follicles with loss of some protactile hair from cheeks, lips, skin and eyelashes, loss of finger- and toenails, streaks on the ocular surface of the eyelid, occlusion of the external ear canal, enlargement of the Meibomian

glands, gastrointestinal disorders and depression (Moore et al. 1979). Monkeys receiving 5 µg.kg-diet^{-1} over 180 d (or until death) showed many of the same signs (McNulty et al. 1982).

Whereas with PCDDs rodents generally show few external signs of toxicity other than weight loss (NRCC 1981a), the PCDFs elicit a variety of toxic responses in rodents. Guinea pigs that received single oral (gavage) doses of 2,3,7,8-T_4CDF (10 µg.kg-BW^{-1}), 2,3,7,8-T_4CDF (15 µg.kg-BW^{-1}) or 2,3,4,7,8-P_5CDF (10 µg.kg-BW^{-1}) developed a spectrum of progressive signs including rough, soiled hair coat, listlessness and dehydration (Moore et al. 1979). Rats fed 10 mg.kg-diet^{-1} of a PCDF mixture for three weeks developed chloracne-like lesions on the ears (Oishi et al. 1978). Mice dosed orally, i.p. or subcutaneously with a single dose of >10 mg.kg-BW^{-1} of a PCDF mixture showed no toxic response except slight weight loss during the first week, but after four weeks showed conjunctivitis, partial alopecia, thickening of the dermis and erosions of the face and neck (Nishizumi 1978). Exophthalmos, corneal opacities and intraorbital hemorrhages were observed in some of the mice given higher doses (up to 100 mg.kg-BW^{-1}, i.p.).

As a result of several major accidents involving PCDFs, there exists an extensive literature documenting signs and symptoms in humans (see Section 7.3). Unfortunately, other toxic compounds were always present so that it is not known if the effects were specific to PCDFs.

7.2.2 BIOCHEMICAL EFFECTS

7.2.2.1 Enzyme Induction - Aryl Hydrocarbon Hydroxylase (AHH)

Like 2,3,7,8- and other PCDDs (NRCC 1981a), 2,3,7,8- and other PCDF homologues have been shown to induce the activity of hepatic enzyme systems. The microsomal monooxygenases, which catalyze the conversion of lipophilic organic compounds to more polar metabolites, have been extensively studied. Only aryl hydrocarbon hydroxylase (AHH) has been investigated with respect to the PCDFs.

AHH represents the activity of cytochrome P-448 (alternative nomenclature P_1-450). Poland and Glover (1973) and Araki (1974) first reported that certain PCDFs are potent inducers of AHH. Poland and Glover (1973) observed that the potency of various PCDFs and PCDDs in inducing AHH activity appeared to parallel their toxic potency. Like 2,3,7,8-T_4CDD (NRCC 1981a), the PCDFs appeared to induce in the same way as classic polycyclic aromatic hydrocarbon inducers such as 3-methylcholanthrene (3-MC). Some congeners are much more potent than 3-MC as an inducer but less potent than equivalent PCDD congeners (e.g.

see Poland and Glover 1973; Araki 1974; Poland et al. 1976b; Kawana and Hiraga 1978; Nagayama et al. 1983).

Poland et al. (1976b) administered various single doses of 2,3,7,8-T_4CDF (in 25 µL p-dioxane) to fertilized chicken eggs and C57BL/6J mice. They also administered 2,3,7,8-T_4CDD, 3,4,3',4'-tetrachloroazoxybenzene (T_4CAOB) and p-dioxane as controls. The three compounds induced the same maximum response in chick embryos, approximately a 10-fold increase in activity. A combination of maximally inducing doses did not further increase enzyme activity in the chick embryos, suggesting the compounds may act through similar mechanisms. While 2,3,7,8-T_4CDD was only slightly less potent in the mouse than in the chicken embryo, 2,3,7,8-T_4CDF and 3,4,3',4'-T_4CAOB were much less potent. The authors speculate that this may be due to more rapid metabolic inactivation.

Structure-activity relationships for PCDF and PCDD congeners have been studied in various systems including chick embryo livers, a rat hepatoma cell line and young male rats (Bradlaw et al. 1976; Poland et al. 1976a; Bradlaw and Casterline 1979; Yoshihara et al. 1981; Nagayama et al. 1983). It is now clear that the PCDD and PCDF congeners more potent for AHH induction have two common structural features:

1) halogen atoms occupy at least three of the lateral ring positions (2,3,7 and 8); and
2) at least one ring position is non-halogenated.

There can be vicinal hydrogens in the ring, in contrast to the conditions necessary for retention of congeners in the liver of Yusho patients (see Section 6.1; Masuda and Kuroki 1982).

Yoshihara et al. (1981) reported that of thirteen PCDF congeners tested (Table 7-1), the nine that significantly induced AHH activity in the liver of young male Wistar rats met the above structural criteria. The most potent congeners were 2,3,7,8-T_4CDF and 2,3,4,7,8-P_5CDF, which induced the enzymes at a single dose of 1 µg.kg-BW^{-1}. Congeners with two or fewer chlorine atoms in the lateral ring positions did not induce the enzyme at a single dose of 10 mg.kg-BW^{-1}.

Poland et al. (1979) accumulated some evidence to support the hypothesis that when a 3-MC type inducer such as 2,3,7,8-T_4CDD enters a liver cell, it binds to a cytosolic receptor protein. This protein-ligand complex is then translocated to the nucleus (Okey et al. 1980) where it activates the genes that code for AHH activity. The cytosolic macromolecular-protein receptor has been isolated and binding affinities with dioxin and furan congeners correspond to their inductive potency and toxicity. Cheney (1982) hypothesized that the lateral halogens are directly involved in the mechanism that transforms the receptor to the active state. This hypothesis is based on the finding that lateral

Table 7-1. Relationship between PCDF homologue structures and ability to induce hepatic enzyme toxicity (after Yoshihara et al. 1981).

Congener	No. of Cls in lateral positions	Vicinal Hs in ring	Hepatic enzymes induction
2,8	2	✓	
1,2,7,8	3	✓	✓
1,3,6,7	2	✓	
1,3,6,8	2		
2,3,6,7	3	✓	✓
2,3,7,8	4		✓ (High)
1,2,3,7,8	4		✓
1,2,4,6,8	3		
1,2,6,7,8	3	✓	✓
2,3,4,6,7	3	✓	✓
2,3,4,7,8	4		✓ (High)
1,2,3,4,6,7	3	✓	✓
1,2,3,4,7,8	4		✓

substituents affect receptor binding and AHH induction somewhat differently.

7.2.2.2 Clinical Biochemical Effects

As with 2,3,7,8-T_4CDD (NRCC 1981a), several biochemical parameters are altered in animals receiving toxic doses of the furans (Table 7-2). Information is available specifically for 2,3,7,8-T_4CDF. The data in Table 7-2 reflect dosing with PCDF mixtures and 2,3,7,8-T_4CDF via a variety of routes of administration, as detailed in the footnotes.

7.2.3 HEMATOLOGICAL EFFECTS (including some Immunological Alterations)

Bone marrow hypoplasia has been observed in monkeys exposed to toxic doses of 2,3,7,8-T_4CDF (Moore et al. 1979; McNulty et al. 1982), although peripheral erythrocyte and leukocyte counts remained normal. There was no decrease in the leukocyte count in Sprague-Dawley (SD) male rats fed a mixture of PCDFs (average Cl number = 4.7) at up to 10 mg.kg-diet^{-1} for 27 d. However, total serum protein and γ-globulin were significantly reduced at 10 mg.kg-diet^{-1} while α- and β-globulin were significantly increased at 1 mg.kg-diet^{-1}. Albumin, α_2-globulin and the albumin-globulin ratio did not differ between treated and control groups (Oishi 1977). Hemoglobin and hematocrit values were decreased in SD rats fed a PCDF mixture at 1 mg.kg-diet^{-1} for 4 weeks (Oishi et al. 1978).

7.2.4 PATHOLOGY

Pathological findings for a variety of mammalian species are summarized in Table 7-3. Some differences among species were apparent, as in the case with the PCDDs (NRCC 1981a). All species studied following administration of either 2,3,7,8-T_4CDF, or PCDF mixtures showed a loss in body weight, liver, kidney and thymus abnormalities and bone marrow hypoplasia. These changes were also observed when 2,3,7,8-tetrabromodibenzofuran or 2,3,4,7,8-P_5CDF was administered to guinea pigs (Moore et al. 1979).

7.2.5 ACUTE LETHALITY

Here, we define acute lethality as death within one month from administration of the toxic compound. As with the TCDDs, the time to death after a lethal dose of PCDF is relatively long (average 12 to 23 d). The latent period does not vary consistently with the magnitude of the dose. For example, guinea pigs died an average of 15 d after 2,3,4,7,8-P_5CDF was administered as a single dose of 10 µg.kg-BW^{-1}

Table 7-2. Summary of clinical biochemical changes due to PCDF toxicity[α].

Parameter	Species			
	Rat	Monkey	Chicken	Mouse
Erythrocyte count	NC[β,κ]	NC[γ]		NC[η]
Leukocyte count	NC[β]	↓[δ]	↓[ζ]	↓[η]
Hematocrit	↓[β]	↓[γ]		
Hemoglobin concentration	↓[β]	↓[γ]		
Corpuscular volume	↓[β]			
Serum calcium		↓[γ]		
Serum albumin	NC[ι]	↓[γ,δ]		
Serum protein	↓[ι], NC[β]	↓[γ]	↓[ζ]	↑[η,θ]
Serum urea nitrogen	↑[β]			
Serum LAP	↑[β]			
Serum triglyceride	↓[β]			
Serum GPT	↑[β]			
Serum GOT	↑[β]	↑[γ,δ]		
Serum cholesterol	↑[β]	↓[γ,δ]		
Serum Na/K	NC[β]			
Total liver lipid	↑[β]			
Testes testosterone concentration	↓[β]			

[α] Data after Moore et al. (1975), Oishi (1977), Oishi et al. (1978), Moore et al. (1979).

[β] 10 μg-PCDF mixture. kg-diet^{-1} for 10 wk.

[γ] 5 μg-2,3,7,8-T_4CDF.kg-diet^{-1} for 2 wk to 6 months.

[δ] 500 μg-2,3,7,8-T_4CDF.kg-BW^{-1} single dose by gavage.

[ε] up to 15 μg-2,3,7,8-T_4CDF.kg-BW^{-1} single dose by gavage.

[ζ] 1 μg-2,3,7,8-T_4CDF.kg-diet^{-1} for 3 wk.

[η] 300 μg-2,3,7,8-T_4CDF.kg-BW^{-1}; 22 doses in 30 d. Gavage.

[θ] Increase due entirely to increased serum globulin, the albumin:globulin ratio was decreased proportionately.

[ι] 10 mg-PCDF mixture.kg-diet^{-1} for 27 d.

[κ] NC = No change.

Table 7-3. Pathological and histological changes due to PCDF toxicity[α].

Effect	Lowest observed-effect level ($\mu g.kg\text{-}BW^{-1}$)[β] Monkey	Rat	Guinea pig	Mice
Loss in body weight	$<500^{\delta,\varepsilon}$; $<0.5^{\delta,\theta}$ (6 wk)	$<50^{\theta}$ (7 d)	$<5^{\delta,\varepsilon,\gamma}$	$300^{\delta,\varepsilon}$ (22 d)$^{\eta}$
Liver abnormality	$>1500^{\delta,\varepsilon}$;	$<1000^{\delta,\zeta,\iota}$; $<50^{\theta}$ (4 wk)	$<5^{\delta,\varepsilon,\gamma}$	$300^{\delta,\varepsilon}$ (22 d)$^{\eta}$
Bile or gall abnormality	$<1000^{\delta,\varepsilon}$; $<0.5^{\delta,\theta}$ (6 wk)			
Spleen atrophy	$<1000^{\delta,\varepsilon}$			
Thymus atrophy	$<500^{\delta,\varepsilon}$; $<0.5^{\delta,\theta}$ (6 wk)	$<50^{\theta}$ (4 wk); $<1000^{\delta,\zeta,\iota}$	$<5^{\delta,\varepsilon,\gamma}$; $<0.5^{\delta,\varepsilon}$	$300^{\delta,\varepsilon}$ (22 d)$^{\eta}$
Bone marrow hypoplasia	$<1000^{\delta,\varepsilon}$; $<0.5^{\delta,\theta}$ (6 wk)		$<10^{\delta,\varepsilon,\gamma}$	
Stomach or intestinal damage	$<1000^{\delta,\varepsilon}$; $<0.5^{\delta,\theta}$ (6 wk)	$<50^{\theta}$ (4 wk)		
Decreased prostate weight		$<50^{\theta}$ (4 wk)		
Brain edema		$<50^{\theta}$ (4 wk)		
Heart abnormality		>50 and $<500^{\theta}$ (4 wk)		
Hyperplasia of epithelial cells in ureter, bladder or renal pelvis or kidney abnormality	$>1500^{\delta,\varepsilon}$	>50 and $<500^{\theta}$ (4 wk)	$<10^{\delta,\varepsilon,\gamma}$	

[α] Data after Oishi (1977), Oishi et al. (1978), Moore et al. (1979), Yoshihara et al. (1981), McNulty et al. (1982). PCDF mixture unless specified.

[β] Single dose unless a time is specified for dose.d^{-1}.

[γ] 2,3,4,7,8-P_5CDF and 2,3,7,8-T_4BDF caused same effect at similar doses.

[δ] 2,3,7,8-T_4CDF.

[ε] Gavage or oral.

[ζ] i.p.

[η] 22 doses in a 30 d period.

[θ] Dose converted from $\mu g.kg\text{-}diet^{-1}$.

[ι] 2,3,4,6,7-P_5CDF and 2,3,4,7,8-P_5CDF caused similar effect at 1 $mg.kg\text{-}BW^{-1}$; 1,2,3,7,8-P_5CCDF and 1,2,3,4,7,8-H_6CDF caused the same effect at 10 $mg.kg\text{-}BW^{-1}$; 2,3,6,7-T_4CDF and 1,2,3,4,6,7-H_6CDF affected thymus weight slightly at 1 $mg.kg\text{-}BW^{-1}$; 1,2,7,8-T_4CDF and 1,2,6,7,8-P_5CDF had no effect on thymus weight at 1 $mg.kg\text{-}BW^{-1}$.

(i.p.) and 9 d after 15 µg.kg-BW^{-1} (i.p.). When 2,3,7,8-T_4CDF was administered at the same doses, the times to death were 12 and 14 d respectively. The latent period is also quite similar in different species. However, LD_{50} values differ dramatically depending on the species, the homologue and the route of administration (Table 7-4).

7.2.6 DERMAL STUDIES

Few studies of the dermal toxicity are available for PCDFs and thus dermal toxicity cannot be compared with oral toxicity. The LD_{50} for a PCDF mixture (42% T_4CDF, 54% P_5CDF, 4% H_6CDF) administered subcutaneously to male and female mice is more than 0.2 g.kg-BW^{-1} (Nishizumi 1978). A mixture of T_4CDFs and P_5CDFs applied to the inside of rabbits ears (0.5, 0.15, 0.025, 0.005 µM.cm^{-2}) caused redness on local skin, followed by thickening of the skin within one to two weeks. Slight hyperkeratosis was observed after 1 week at the highest dose and after 2 weeks at 0.15 µM.cm^{-2} (Nishizumi et al. 1975). There are no studies on dermal absorption.

7.2.7 CHRONIC TOXICITY

No animal study using a PCDF has extended over more than six months. There are no data on the chronic effects of the PCDFs. The reader is referred to the discussion of the PCDDs in NRCC (1981a) for the types of effects that could be expected.

7.2.7.1 Carcinogenicity

No data are available on the carcinogenicity of the PCDFs. It is relevant to note that 2,3,7,8-T_4CDD is either a primary or secondary carcinogen in rats and mice and the apparent NOEL observed in the available studies is 0.001 µg.kg-BW^{-1}.d^{-1}. H_6CDD has also been shown to be carcinogenic, but has about 1/36th the potency of 2,3,7,8-T_4CDD (NRCC 1981a).

7.2.8 TERATOGENIC AND REPRODUCTIVE EFFECTS

While 2,3,7,8-T_4CDD and some other homologues have been shown to be fetotoxic and teratogenic in several types of laboratory mammals at very low doses (NRCC 1981a), no studies have directly investigated the reproductive toxicity of the PCDFs.

The PCDFs are known to be efficiently transferred to fetuses and sucklings in mice (see Section 6.1.1; Nagayama et al. 1980). Based on their similarities to the PCDDs, the PCDFs are quite likely to elicit adverse reproductive effects. In NRCC (1981a), the teratogenic NOEL for

Table 7-4. Single dose LD_{50}s for PCDFs administered to various species by gavage, orally or i.p.

Species	LD_{50} µg.kg-BW^{-1}	Average time to death (d)	Reference
Hartley Guinea Pigs	2,3,7,8-T_4CDF[α]		Moore et al.
	> 5[β], < 10[β]	12-15	(1975, 1979)
	7	< 30	McKinney and McConnell (1982)
	2,3,7,8-T_4CDF[η]		Moore et al.
	> 1.6[β], < 15[β]	15	(1979)
	2,3,4,7,8-P_5CDF		Moore et al.
	> 3[β], < 10[β]	16	(1979)
	2,3,4,6,7,8-H_6CDF		McKinney and
	120	< 30	McConnell (1983)
CF1 Mice	PCDF mixture[ε]		Nishizumi (1978)
(male)	184,000[γ]	16	
(female)	414,000[γ]	?	"
(male & female)	< 60,000[δ]	< 21	"
Rhesus Monkeys	2,3,7,8-T_4CDF[ζ]		Moore et al.
(female)	1000[β]	23	(1979)

[α] 88% pure, the remainder mainly P_5CDFs.

[β] Gavage.

[γ] Oral.

[δ] i.p.

[ε] 42% T_4CDFs, 54% P_5CDFs, 4% H_6CDFs.

[ζ] > 98% pure.

[η] 2,3,7,8-tetrabromodibenzofuran.

2,3,7,8-T_4CDD in mice is reported to be 0.1 µg.kg-BW^{-1}.d^{-1} and the NOEL for H_6CDD is less than 100 µg.kg-BW^{-1}.d^{-1} in rats. The NOEL for adverse reproductive effects of 2,3,7,8-T_4CDD is 0.001 µg-2,3,7,8-T_4CDD.kg-BW^{-1}.d^{-1} in rats and is estimated to be 0.0016 µg.kg-BW^{-1}.d^{-1} in monkeys. These NOELS could, in the absence of more appropriate data, be used as a first approximation of the toxicity of 2,3,7,8-T_4CDF.

7.2.9 MUTAGENIC EFFECTS AND CHROMOSOMAL ABERRATIONS

Schoeny (1982) tested four PCDF homologues (2,9-D_2CDF, 2,6-D_2CDF, 2,3,7,8-T_4CDF, O_8CDF) and dibenzofuran for mutagenicity in the Ames reversion assay using two strains of Salmonella typhimurium histidine auxotrophs (TA98 and TA100). None of the homologues showed a mutagenic effect over a wide dose range, whether or not microsomal extracts were included. In three other Salmonella typhimurium strains (TA1535, TA1537 and TA1978), 2,3,7,8-T_4CDF was also found not to be mutagenic (Schoeny 1979).

Inoue et al. (1979) reported on the effect of a mixture of PCDFs on sister-chromatid exchanges (SCE) in cultured chinese hamster lung (CHL) cells. They found a dose-related increase in SCE per cell, which was statistically significant when the PCDFs were added to the medium at 0.1 or 1 µg.mL^{-1}.

2,3,7,8-T_4CDF has been reported to promote the mutagenic activity of benzopyrene when used as a hepatic enzyme inducer (Schoeny 1982). With 5 µg-benzopyrene per plate of TA100 Salmonella typhimurium, the number of revertants (mutations) was dependent on the type of microsomal extract included. Extract from uninduced rats produced minimal activation while extracts from rats treated with 90 µg of 2,3,7,8-T_4CDF.kg^{-1} produced 5 revertants per µg of microsomal protein. This was equivalent to the effect of extract from rats treated with 40 mg of 3-methylcholanthrene, a potent microsomal enzyme inducer.

7.2.10 IMMUNOTOXICITY

Atrophy of the thymus, and adverse effects on other lymphatic tissue such as bone marrow or spleen have been observed as part of the toxic response to PCDFs in almost all the species studied, although peripheral erythrocyte and leukocyte counts remained normal (Sections 7.2.3 and 7.2.4). These observations, together with indications of immunotoxicity for similar compounds such as the TCDDs, have led to a variety of studies specifically designed to investigate the effects of PCDFs (particularly 2,3,7,8-T_4CDF) on the immune system.

One strategy has been to assess the impact of PCDFs on the susceptibility of animals to various assaults on the immune system. Oishi and Hiraga (1980) compared the effects of a PCDF mixture (12%

T_4CDF, 88% P_5CDF), a PCDD mixture (33% H_7CDD, 66% O_8CDD) and a PCB mixture on the susceptibility of four-week-old pathogen-free male ICR/JCL mice to an endotoxin (*E. coli* lipopolysaccharide). The mice were dosed by gavage with 0.10 or 100 $\mu g.kg\text{-}BW^{-1}.wk^{-1}$. Two days after the final treatment the mice were given the endotoxin i.p. and sensitivity to the endotoxin was determined as mortality within 24 h. As reported in Table 7-5, a possibly dose-related increase in mortality was observed with the PCDF and PCDD mixtures. The PCDF mixture's greater potency was possibly due to the different congeners present. A significant decrease in thymus weight was observed only for the PCDF-treated mice.

Luster *et al.* (1979a, b) attempted to assay the impact of PCDF on cell-mediated and humoral immune function by measuring thymidine incorporation into cells during lymphocyte blastogenesis *in vitro*. Certain phytomitogens generally induce thymus-derived lymphocytes (T-cells) preferentially while others preferentially induce bursa-equivalent derived lymphocytes (B-cells). The fundamental limitations of this *in vitro* system are mentioned in NRCC (1981a).

Female Hartley guinea pigs received 2,3,7,8-T_4CDF by gavage at 0, 0.05, 0.17, 0.5 or 1 $\mu g.kg\text{-}BW^{-1}.wk^{-1}$ for six weeks. Weight gain was suppressed in almost all the dosed animals and the suppression trends were dose-related, although none of the groups showed a statistically significant reduction. Some of the animals in the 1 $\mu g.kg\text{-}BW^{-1}.wk^{-1}$ dose group died. Total leukocyte counts were normal in all groups tested and histologically, spleens and thymuses from all dosage groups appeared normal. Ratios of thymus to body weight were significantly reduced in the 0.5 and 1.0 $\mu g.kg\text{-}BW^{-1}.wk^{-1}$ groups but there was no change in spleen:body weight ratios. Ten days after the final dose, splenic lymphocytes were harvested and cultured with the T-cell mitogens phytohemaglutinin (PHA) and Concanavalin A (Con A) or the B-cell mitogen lipopolysaccharide (LPS). PHA and LPS significantly reduced the blastogenic response, as measured by ^3H-thymidine uptake, in the 0.5 and 1.0 $\mu g.kg\text{-}BW^{-1}.wk^{-1}$ dose groups. This suggests 2,3,7,8-T_4CDF inhibited both the B-cell and T-cell response.

Luster *et al.* (1979a, b) also tested cell-mediated effective mechanisms by determining the ability of antigen-sensitized cells to release the migration inhibition factor (MIF), which prevents macrophage migration *in vitro*. They found a dose-related inhibition in all the dosed groups, although the inhibition was statistically significant only in the 0.5 $\mu g.kg\text{-}BW^{-1}.wk^{-1}$ group (Table 7-6).

They also investigated the effect of PCDFs on cell-mediated delayed hypersensitivity reactions. This type of test involves the sensitization of an animal to an antigen, followed by a topical challenge (usually on the ear) 7 to 14 days later. A dose-related effect was noted at all dose levels, statistically significant in the 0.5 $\mu g.kg\text{-}BW^{-1}.wk^{-1}$ dose group (Table 7-6).

Table 7-5. Effect of a PCDF mixture on the susceptiblity of ICR/JCL mice to endotoxin (after Oishi and Hiraga 1980).

Dose $\mu g.kg\text{-}BW^{-1}.wk^{-1}$	Mortality[α]			
	Endotoxin (μg per mouse)			
	0	50	250	500
0	0	0	0	0
10	0	0	0	2
100	0	1	3	3

α Number of mice that died of the 10 mice in each dose group.

Table 7-6. Impact of 2,3,7,8-T_4CDF on immune parameters in Hartley guinea pigs (after Luster *et al.* 1979a, b).

Dose $\mu g.kg\text{-}BW^{-1}.wk^{-1}$	Mitogen stimulation index[α] PHA	Con A	LPS	Macrophage inhibition index[β]	Delayed hypersensitivity index[γ]	Antibody titre
0	27.3±3.7	17.5±2.4	16.6±2.1	6.0±1.8	1.9±0.4	Normal
0.05	23.5±4.9	12.1±1.5	12.6±2.5	3.9±0.5	1.5±0.1	Normal
0.17	26.6±7.0	17.5±5.4	11.9±2.5	3.1±1.2	1.5±0.1	Normal
0.5	17.5±3.9[δ]	14.3±3.5	9.6±2.5	3.0±0.6[δ]	1.4±0.1	Slight decrease (~ 20%)
1.0	16.3±3.11[ε]	11.9±3.1	8.2±1.6	Not determined		

[α] The ratio of the mean ^{3}H-thymidine uptake in mitogen stimulated cultures to that in non-stimulated cultures.

[β] The ratio of the mean migration area in stimulated cultures to that in non-stimulated cultures.

[γ] The ratio of the response in the challenged ear to that in the control ear.

[δ] $p < 0.05$.

[ε] $p < 0.1$.

These data suggest that 2,3,7,8-T_4CDF, like 2,3,7,8-T_4CDD (NRCC 1981a), is capable of suppressing cell-mediated immunity.

Luster et al. (1979a, b) further evaluated the impact of 2,3,7,8-T_4CDF on the humoral immune response by quantitating serum IgG levels and the antibody response following immunization with bovine gammaglobin (BGG). Serum IgG levels were normal in all groups. A slight decrease in antibody titre was observed only in the 0.5 $\mu g.kg\text{-}BW^{-1}.wk^{-1}$ group (the highest dose group tested) 4 weeks after immunization. This lack of effect is not necessarily inconsistent with the decreased response to LPS mitogen as the antibody response to LPS may be independent of its mitogenic properties. Alternatively, as the authors suggest, their study may be insensitive to subtle alteration in humoral immunity.

In all of the above experiments, the immune effects of 2,3,7,8-T_4CDF were strikingly similar to those of 2,3,7,8-T_4CDD.

Vecchi et al. (1983) compared the immunosuppressive effects of 2,3,7,8-T_4CDF and 2,3,7,8-T_4CDD in C57BL/6 and DBA/2 mice. The compounds were administered i.p. or orally at doses up to 900 $\mu g.kg\text{-}BW^{-1}$. Even the highest 2,3,7,8-T_4CDF doses did not affect C57BL/6 mice body weight or the total number of peritoneal cells or macrophages, but 180 $\mu g.kg\text{-}BW^{-1}$ significantly reduced thymus weight and lowered the recoverable number of spleenocytes. The effects on humoral antibody production were investigated in C57BL/6 mice by dosing the mice 7 d before antigenic stimulation. Figure 7-1 shows the dose-response curve for both 2,3,7,8-T_4CDD and 2,3,7,8-T_4CDF. Results were similar for oral and i.p. administration. The time course of immunosuppression was also investigated. While the 2,3,7,8-T_4CDF-induced inhibition had completely disappeared 42 days after doses of 2,3,7,8-T_4CDD and 2,3,7,8-T_4CDF that were equipotent on day 7, the 2,3,7,8-T_4CDD-induced inhibition was then still strong. The authors speculate that this difference may simply reflect the different whole body half-lives for elimination (2 d for 2,3,7,8-T_4CDF and 17 d for 2,3,7,8-T_4CDD).

Because of the known differences in 2,3,7,8-T_4CDD induced immunosuppression in C57BL/6 and DBA/2 mice (Garattini et al.1982), which also differ in the level and/or affinity of the specific receptor responsible for AHH induction by TCDD (Poland and Glover 1975) and in the in vitro binding of 2,3,7,8-T_4CDD to thymic cytosol (Okey et al. 1979), 2,3,7,8-T_4CDF and 2,3,7,8-T_4CDD were investigated in the two strains. Approximately 85% inhibition of humoral antibody production was observed to be induced in C57BL/6 mice while only 40% inhibition was observed in DBA/2 mice by the two compounds at doses of 180 $\mu g.kg\text{-}BW^{-1}$ and 6 $\mu g.kg\text{-}BW^{-1}$ respectively.

When Rizzardini et al. (1982) administered 2,3,7,8-T_4CDF (10 $\mu g.kg\text{-}BW^{-1}$) with 2,3,7,8-T_4CDD (1.2 $\mu g.kg\text{-}BW^{-1}$) as a single dose i.p. to

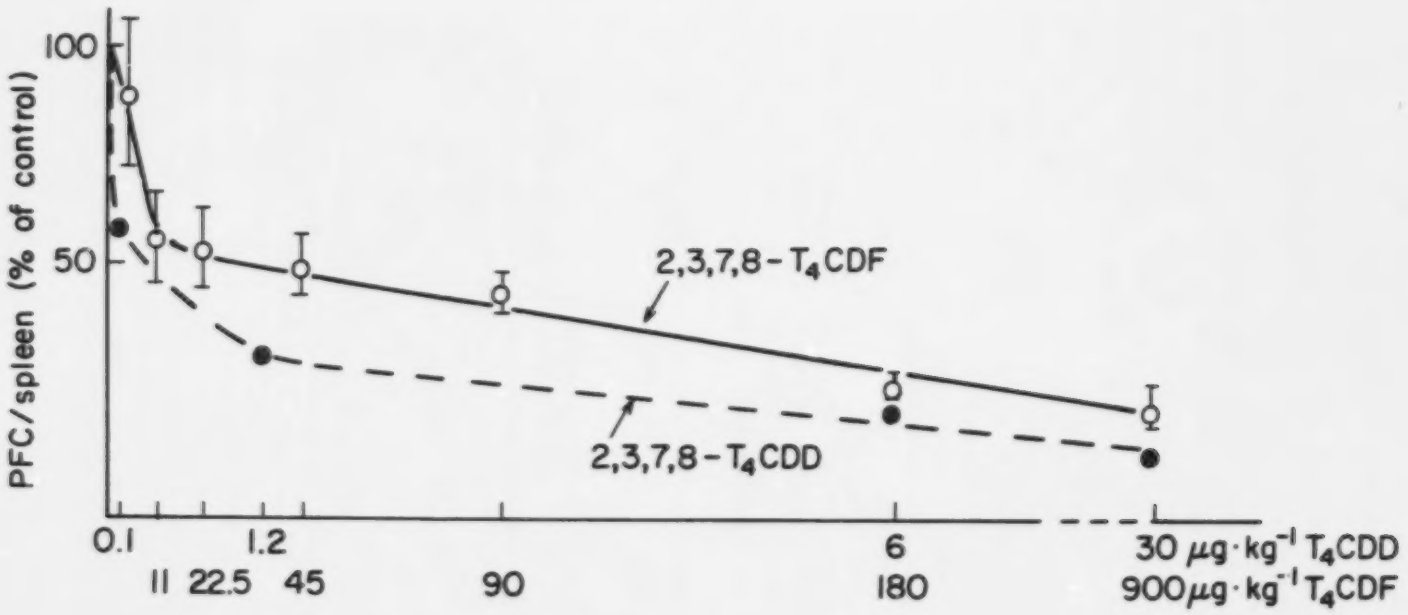


Figure 7-1. Dose-response effect on humoral antibody production in C57BL/6 mice of 2,3,7,8-T_4CDD and 2,3,7,8-T_4CDF given i.p. 7 d before the antigen (4 x 10^8 SRBC)[α] (after Vecchi et al. 1983).

α Test was performed 5 d after antigenic stimulation.

β Plaque forming cells.

C57BL/6 mice, they observed that the immunosuppression decreased significantly from that induced by 2,3,7,8-T_4CDD alone.

7.3 EFFECTS ON HUMANS

In Japan, in 1968, mass consumption of a cooking oil (Kameni Rice Oil) contaminated with PCB (~2500 $\mu g.L^{-1}$ of Kanechlor 400 (KC 400) previously used as a heat transfer agent) caused an outbreak of a disease known as "Yusho" which affected over 1000 people (Hayabuchi et al. 1979; Kuratsune et al. 1972). Later this disease was attributed at least in part to the 40 tri to hexa PCDFs (including 2,3,7,8-T_4CDF, 2,3,4,7,8-P_5CDF and 2,3,4,6,7-P_5CDF as major components) which also contaminated the oil at levels up to 5 $\mu g.L^{-1}$ (Nagayama et al. 1976; Rappe et al. 1977; Buser et al. 1978c; Masuda et al. 1982). It has been estimated that Yusho patients consumed an average of 3.4 mg PCDF (48 $\mu g.kg\text{-}BW^{-1}$ for a 70 kg man) (Hayabuchi et al. 1979); 2,3,4,7,8-P_5CDF was the major component. PCDFs have been found in tissue from Yusho patients at $ng.kg^{-1}$ levels (Chapter 6). The initial toxic clinical symptoms included swelling of the eyelids and eye discharge, acne, swelling of limbs, digestive disruptions, numbness and other neurological signs, and skin pigmentation (Kuratsune et al. 1972). In 1979, a similar episode also involving PCB contaminated rice oil was reported from Taiwan; about 200 people were involved (Wong et al. 1982). The levels of PCBs (Kanechlor 400 and 500) and PCDFs were somewhat lower in the Taiwan oil, about 100 and 0.1 $\mu g.L^{-1}$ respectively (Masuda et al. 1982), but the isomer spectrum was similar.

The occurrence and severity of the toxic response in Japan correlated with the estimated amount of oil consumed (Kuratsune et al. 1972; Hayabuchi et al. 1979) but not at all with the amount of oil consumed per kg-BW per day (Hayabuchi et al. 1979). This may reflect the ability of the highly-chlorinated PCBs and PCDFs to accumulate in the body. It is interesting to note that the latent period before the onset of toxic symptoms showed a negative correlation with the amount of oil consumed per kg per day, in accord with general principles of toxicology but in contrast to other aspects of PCDF toxicology, such as the latent period before death following administration of toxic doses to laboratory mammals (see Section 7.2.5).

The signs and symptoms observed in these human patients resemble those described for laboratory primates (Section 7.2.1), including mucocutaneous pigmentation, acne, deformed nails and dry skin (Wong et al. 1982).

Fischbein et al. (1982) reported that in 326 capacitor manufacturing workers a high prevalence (37%) of dermatological abnormalities was found. The symptoms included erythema, dryness and thickening of the skin, hyperpigmentation, rash, swelling and acne. When a subgroup of the most affected workers with skin abnormalities

(thought to be related to PCB exposure in particular) were compared with non-affected workers, a significant difference was found between the mean concentrations of the higher PCB homologues. PCDF concentrations were not investigated in this study but capacitor workers could be subject to high PCDF exposure (Section 5.2.2).

It is not possible to draw firm conclusions about the effect of PCDFs on humans from incidents involving PCBs because in such cases the people are also exposed to the PCBs per se and other contaminants such as polychlorinated quaterphenyls (PCQs) in the case of Japanese Yusho. Although the PCDFs are generally much more toxic than the other chemicals, they are usually present in relatively small amounts. In Yusho oil the ratio of PCDF:PCQ:PCB was 1:160:500 (Kashimoto et al. 1981).

Hori et al. (1982) attempted to clarify the effects of PCBs, PCQs and PCDFs by administering purified PCBs ("free" of PCDF), PCB contaminated with PCDFs, and PCQs, orally to monkeys (Macaca fascicularis) at 5 $mg.d^{-1}$ for 20 weeks. In the groups fed purified PCB, immunosuppression and histopathological changes of the liver were observed. In the group receiving contaminated PCBs, there were more marked decreases in body weight, as well as immunosuppression and fatty liver and histopathological changes; in addition, hair loss, acne eruptions, edema of the eyelid, congestion of the Meibomian gland and other characteristic dermatological findings of Yusho disease were noted. In the group fed PCQs, there was immunosuppression as well as enlarged liver but no decrease in body weight or skin abnormalities. These results suggest that the PCDFs were probably largely responsible for the symptoms observed in Yusho disease.

7.4 TOXICITY TO BIRDS

In 1957, millions of broiler chickens died in the eastern and midwestern parts of the United States from toxic components in certain fats incorporated in their feed. The characteristic symptoms included hydropericardium, ascites, anasarca, subcutaneous edema and liver necrosis. The toxic components were identified as dioxins and furans (Firestone 1973). Kohanawa et al. (1969) associated an epizootic of chick edema disease in Japan with rice bran contaminated with Kanechlor 400 which has also been identified as the etiologic agent of Yusho disease in humans.

Reproductive failure resembling chick edema disease was observed in certain fish-eating birds of the Great Lakes during the early 1970s (Gilbertson 1983). Analysis of stored herring gull eggs from the Great Lakes, including Saginaw Bay, revealed the presence of significant quantities of 2,3,7,8-T_4CDD, 2,3,7,8-T_4CDF and a number of other identified and unidentified contaminants (Norstrom et al. 1982; NRCC 1981a; Gilbertson 1983; Gilbertson and Fox 1983). The relative

importance of any individual contaminant in causing chick edema disease is still being assessed.

Symptoms approximating chick edema disease have been produced experimentally with certain PCDDs and PCDFs, including 2,3,7,8-T_4CDD (NRCC 1981a) and 2,3,7,8-T_4CDF (McKinney et al. 1976). When 5 μg-2,3,7,8-T_4CDF.kg-BW^{-1}.d^{-1} was administered by gavage for 21 days to 1-day-old white leghorn cockerels, the chicks showed decreased food consumption and weight gain, and died after an average of 11.5 d. Hydropericardium, subcutaneous edema and ascites were observed. Liver weights were normal and only minor lesions were observed. There was involution of the thymus and depletion of lymphatic cell types in the spleen and thymus. Birds dosed at 1 μg-2,3,7,8-T_4CDF.kg-BW^{-1}.d^{-1} also showed decreased food consumption and weight gain, reduced spleen weight, inflammatory changes on the pericardical surface of the heart, and a few chicks showed endocardial edema. One bird (of 6 in the dose group) died on day 19.

Goldstein et al. (1976) reported that 1-day-old white leghorn cockerels given 1 or 5 μg-2,3,7,8-T_4CDF.kg-BW^{-1}.d^{-1} by gavage for 3 weeks showed significant increases in microsomal protein and cytochrome P_{450} (AHH). There were no apparent changes in hepatic ALA synthetase activity, porphyrin accumulation or glucuronyl tranferase.

The oral LD_{50} for white leghorn male chicks has been established as >1 and <5 μg-2,3,7,8-T_4CDF.kg-BW^{-1}.d^{-1} (Moore et al. 1975; McKinney et al. 1976).

7.5 TOXICITY TO AQUATIC ORGANISMS

There are very few data on the toxicity of the PCDFs to aquatic organisms. Juvenile Atlantic salmon, fed a PCDF mixture in the diet (2.7 D_2CDF, 5.7 T_3CDF, 2.8 T_4CDF, 9.1 O_8CDF μg.g-diet^{-1}), had a median mortality of 120 ± 30 d (Zitko and Choi 1973). No mortality was observed when immature brook trout were given a single dose of 2,8-D_2CDF at levels as high as 122 mg.kg-BW^{-1} (Zitko et al. 1973). Zitko et al. (1973) indicated that after dietary dosing, tri and tetra PCDFs were retained preferentially to 2,8-D_2CDF in fish liver and muscle, with greatest retention in the liver.

Fingerman and Fingerman (1977) reported that O_8CDF at a concentration of 16 x 10^{-6} μg.L^{-1} in the aquarium water slightly inhibited the rate of molting of the fiddler crab Uca pugilator.

APPENDIX I.0 CRITERIA FOR ASSESSING AND RATING PUBLICATIONS ON PCDF ANALYTICAL METHODS

I.1 CRITERIA FOR ASSESSING AND RATING PUBLICATIONS

It is axiomatic that an analytical method provide reliable data. To insure that it will, certain stringent criteria must be met. Proven methodology is particularly important when determining residues of toxic compounds, such as the PCDFs, because their presence in foods, etc., at any level has extreme toxicological, economic and political ramifications. In reviewing and evaluating the methods described here, we have freely used the planning and evaluation guidelines described by ACS (1980), Crummett (1979) and Gunther (1970).

Each method was critically examined for the presence and depth of: sampling plan, chemical work-up, recovery studies, limits of detection and quantitation, extent of validation, confirmation, and collaborative studies, in terms of its intended purpose, i.e. to serve as a method specific for isomers or a screening procedure capable of determining homologues only. To assist in this evaluation, a rating scheme was devised to reflect the degree of confidence in the residue data generated by the application of a given method.

Table I-1 lists each point rating and its essential elements. A rating of 1 is given to a method fully evaluated by collaborative studies using good laboratory practices and audited under a sound quality assurance program. The official methods of the Association of Official Analytical Chemists are examples of procedures that approach this level.

A rating of 2 indicates that the method is completely isomer specific, is validated through extensive recovery studies, has interferences minimized by extensive and multiple clean-up procedures, but lacks collaborative evaluation and assumes that confirmation has taken place.

A rating of 3 indicates that the method is not completely isomer specific, has recovery studies partially complete, has interferences partially minimized through chemical cleanup, but lacks collaborative evaluation and assumes confirmation.

A rating of 4 indicates the method is not isomer specific, is open to interferences as cleanup is not extensive, is partially validated as to recovery and is capable of detecting PCDFs down to a specified level, but lacks collaborative evaluation and confirmation.

A rating of 5 indicates the method is inadequately documented but contains most of the essential elements noted under a rating of 4.

Table I-1. Criteria for rating publications on PCDF analytical methods.

Point Rating	Essential Elements
1 (highest)	Complete quality assurance as described by ACS (1980). An ideally developed, evaluated method including collaborative studies.
2	Isomer specific, extensive recovery studies, interferences removed and separation achieved through extensive chemical work-up, but lacks collaborative evaluation and assumes confirmation.
3	Incompletely isomer specific, some recovery studies, interferences partially removed and partial separation achieved through chemical work-up, lacks collaborative evaluation and assumes confirmation.
4	Essentially a screening method for most homologues, interferences partially removed and partial separation through limited chemical work-up, lacks collaborative evaluation and assumes confirmation.
5	Same as 4, except inadequately documented for recovery, cleanup, etc.
6	Insufficient for the present "state-of-the-art".

Finally, a rating of 6 indicates that the method is totally inadequate for providing data as required by the present "state-of-the-art". Examples of such methods are those used a decade or more ago.

This rating scheme is highly subjective and therefore can easily reflect an individual's bias. However, this can be overcome by using group participation to obtain a consensus; some validity has been achieved with this relative assessment. Additionally, one must be continually aware that a procedure having a low rating is not necessarily a poor or bad method per se. For example, negative results of screening methods with known limits of detection (LOD) are excellent data and are often totally reliable. It is the end use that ultimately determines whether a method ranked 1-4 is adequate. For example, methods ranked at level 3 are perfectly adequate for screening but generally unacceptable for most regulatory purposes. Many methods prove to be excellent procedures when validated. However, the majority of methods reviewed in this critique have been inadequately validated and thus are rated 3,4 or 5. Therefore, collaborative studies between laboratories are essential to verify the confidence of reported data.

APPENDIX II.0

TABULAR ABSTRACTS OF METHODS REVIEWED FOR BIOLOGICAL AND ENVIRONMENTAL MATRICES

Table II-1. Methods reviewed for biological and environmental matrices.

Reference, Critique, Rating	METHOD; BASIC COMPONENTS						VALIDATION OF METHOD			
	Matrix	Extraction	Cleanup	Separation and Detection System	Detection Limits	Confirmation	Level of Fortification	N	% Recovery	± S.C. or C.V.
Rappe et al. (1981) -used the procedure of Stalling et al. (1980) Rating = 4	snapping turtle and grey seal adipose tissue 50-100 g	-grind with sodium phosphate -apply as top segment in first chromatography system	-potassium silicate/silica gel segmented chrom. -carbon dispersed on glass fibres chrom.; elute with toluene to recover PCDFs, PCDDs exchange for hexane -H_2SO_4/silica gel in tandem with cesium silicate chrom. -alumina chrom.	-GC/MS 50 m glass capillary columns of OV-17 and Silar 10C; use 2 µL aliquots containing 0.1 and 0.8 g of the turtle or grey seal extract, respectively -NCI mass spectra m/e 80-500	0.2-0.5 pg of 2,3,7,8 T_4CDF and O_8CDF, respectively	-MS of M⁻ ions, intensity Cl isotopes - GC retention times on 2 columns	-	-	-	-

Table II-1. (Cont'd)

Reference, Critique, Rating	METHOD; BASIC COMPONENTS						VALIDATION OF METHOD			
	Matrix	Extraction	Cleanup	Separation and Detection System	Detection Limits	Confirmation	Level of Fortification	N	% Recovery	± S.C. or C.V.
Stalling et al. (1982) -see Stalling et al. (1979b), Stalling (1980), Ribick et al. (1981) for complete procedure detail Rating = 4	fish, turtle and seal adipose tissue oils	-grind tissue with Na_2SO_4, 1 part tissue 4 parts salt -place as top segment in first clean-up column	-see Smith (1980)	-GC/MS/NCI capillary 50 m OV-17 or Silar 10C coupled to Finnigan 4000 using methane as the reagent gas	1 pg	-NCI/MS -GC/HR capillary columns OV-17; Silar 10C -highly selective cleanup	200 ng.kg^{-1} in 20 g of fish oil PCDF: 2,3,6,8- 2,3,7,8- 1,2,4,7,8- 1,2,3,4,7,8- 1,2,3,4,6,7,9- O_8CDF O_8CDD	4	 81 92 98 104 95 99 91	 1 3 6 4 8 22 16

Table II-1. (Cont'd)

Reference, Critique, Rating	METHOD; BASIC COMPONENTS				Detection Limits	Confirmation	VALIDATION OF METHOD			
	Matrix	Extraction	Cleanup	Separation and Detection System			Level of Fortification	N	% Recovery	± S.C. or C.V.
Stalling et al. (1983) -extraction proc. for fish oil from whole fish not given -detail on cleanup from Stalling et al. (1980) -this procedure rated high clean-up efficiency as reported by Brumley et al. (1981) for TCDD Rating = 4	fish oil from 3-5 whole fish composites	-fish oil dissolved in CH_2Cl_2 at conc. less than 200 $mg.mL^{-1}$	-potassium silicate/silica gel chrom. Eluant: 1. 50 mL CH_2Cl_2 -carbon/glass fibre chrom. Eluant: 1. 200 mL CH_2Cl_2: methanol:benzene, 75:20:5 V/V/V 2. 50 mL toluene 3. 100 mL eluant 1 -replace eluant 2/ toluene with hexane -sulfuric acid/silica gel chrom. in tandem with cesium silicate chrom. Eluant: 1. 4 mL hexane -alumina chrom. Eluant: 1. 10 mL hexane 2. 15 mL 2% CH_2Cl_2 in hexane 3. 15 mL 5% CH_2Cl_2 in hexane 4. 15 mL 8% CH_2Cl_2 in hexane	-GC/MS Finnigan 4023 using NCI and PCI with methane or isobutane -GC column 30 m x 0.25 mm i.d. fused silica capillary coated with DB-5; temp. program 180°C 5 min to 255°C at 3C°/min then 12C°/min to 291°C and held for 10 min	0.5 pg PCDFs using NCI	-GC/MS and GC retention times	-not given for PCDFs	-	50-75	-

Table II-1. (Cont'd)

Reference, Critique Rating	METHOD; BASIC COMPONENTS						VALIDATION OF METHOD			
	Matrix	Extraction	Cleanup	Separation and Detection System	Detection Limits	Confirmation	Level of Fortification	N	% Recovery	± S.C. or C.V.
Ryan and Williams (1983) Rating = 3	human fat 10-20 g	-homogenize tissue, shake twice with 120 mL acetone: hexane, 2:1 and 40 mL water; wash with water	-defat with sulfuric acid -Florisil column chrom.	-reverse phase HPLC -capillary GC/MS in EI mode at low resolution	1 to 4 $\mu g.kg^{-1}$	- 30 m DB-S column at 9000 resolution	-not given for PCDFs	20	52.4 for 2,3,7, 8-T_4CDD	18.6%

Table II-1. (Cont'd)

Reference, Critique Rating	METHOD; BASIC COMPONENTS				Detection Limits	Confirmation	VALIDATION OF METHOD			
	Matrix	Extraction	Cleanup	Separation and Detection System			Level of Fortification	N	% Recovery	± S.C. or C.V.
Rappe et al. (1979b) Rating = 5	human liver	-see Nagayama et al. (1977) and Kuroki and Masuda (1978) -PCDF fractions sent to Switzerland for GC/MS analysis		-GC/MS Finnigan 4000 with EI -GC capillary 50 m x 0.37 mm i.d. coated with OV-17 or Silar 10C -see Buser et al. (1978a) for GC/MS details	-	-GC retention times, 2 columns -m/z values similar to congener standards	-	-	-	-

Table II-1. (Cont'd)

Reference, Critique, Rating	METHOD; BASIC COMPONENTS				Detection Limits	Confirmation	VALIDATION OF METHOD			
	Matrix	Extraction	Cleanup	Separation and Detection System			Level of Fortification	N	% Recovery	± S.C. or C.V.
Dougherty et al. (1980) -outline of methods and results of their screening analysis applied to human and food matrices using GC capillary column/MS NCI separation and detection -clean-up procedures and NCI ionization gases were tailored to the matrix and xenobiotic chemicals being determined e.g. methylene chloride for aliphatic polyhalogen compounds; hydrocarbon/oxygen mixtures for aromatic polyhalides, polynuclear aromatic hydrocarbons i.e. isobutane/O_2 for PCDD's, PCDF's, naphthalenes Rating = 5	adipose tissue 25 mg fish 150 mg or 25 g	-+100 mL 10% H_2SO_4, homogenize in Vitris blender; heat mixture at 80°C for 4 min; steam distill into 2 mL 2,2,4-trimethyl pentane for 1 h; separate organic layer; conc. using Snyder column; analyze an aliquot by NCI; or subject to gel permeation cleanup mix with 2.0 g anhydrous Na_2SO_4 and soxhlet extract with 1:1, hexane: acetone 4 h; conc. to 1.0 mL and cleanup on gel permeation column -mix 25 g with 100 g anhyd. Na_2SO_4 and pack in a glass column; extract with 250 mL CH_2Cl_2	-gel permeation chrom. 25 mg fish 50 cm x 1.0 cm column Bio-Rad SX-8 2.0 mL/min cyclohexane/CH_2Cl_2. U.V. 254 nm detector (or) -gel permeation chrom. aliquot of 25 g extract concentration equivalent 1 g or less on 48 cm x 2.5 cm column of Bio-Bends S-X3; elute with 1:1, cyclohexane:CH_2Cl_2; collect 160-220 mL fraction; conc. to 30 mL -5% carbon AMOCO PX-21 polyurethane from chrom. 1 x 8.5 cm column Eluant: 1. 30 mL ethylacetate 2. 20 mL 4% benzene/ethylacetate 3. 20 mL 20% benzene /ethylacetate 4. 20 mL 50% benzene/ethylacetate 5. reverse flow 30 mL toluene -conc. eluate 5 to 50 µL	-analyze eluate 5 for planar molecules 1. GC/EC 2. MS/NCI (isobutane/oxygen) direct probe: this fraction screens for PCDDs, PCDFs and phthlates using AEI MS-902 and GC/EC analysis but no detail given 3. GC/EI MS, Finnigan 4023	1 pg	-	-	-	-	-

Table II-1. (Cont'd)

Reference, Critique, Rating	METHOD; BASIC COMPONENTS				% Recovery Data							
	Matrix	Extraction	Cleanup	Separation and Detection System	Spike ($\mu g.kg^{-1}$)	T_4CDD β / T_4CDF	P_5CDF	H_6CDF	H_7CDD	H_7CDF	O_8CDD	O_8CDF
Smith (1980) -interference from phthlates was experienced from gas systems, teflon tubing, requiring strict remedial procedures to reduce contamination -this procedure also reported by Stalling et al. (1980) Rating = 4	fish tissue 100 g	-grind tissue, mix with 400 g Na_2SO_4, dry at ambient temp. 12 h; blend to free flowing powder I. Extraction/Clean-up, 3 column in tandem; 1. tissue/Na_2SO_4 over potassium silicate segment over silica gel segment 2. cesium silicate segment over silica gel segment 3. carbon/glass fibre mix -eluant 600 mL C_6H_{12}:CH_2Cl_2, 1:1; then carbon/glass only with, 75 mL C_6H_{12}:CH_2Cl_2, 50 mL CH_2Cl_2:MeOH:benzene, 75:20:5 -discard all eluates	-desorption of aromatic residues from carbon/glass -reverse elute column with 40 mL toluene, conc. to 0.5 mL, replace with hexane II. Fractionation of aromatic residues; 2 chrom. column in tandem 4. cesium silicate segment over H_2SO_4/silica gel 5. alumina -add 0.5 mL hexane solution to column 4 -elute both columns 5 mL hexane -elute column 5 with 5 mL hexane and follow with 15,15,20 mL of 2%, 5% and 8% CH_2Cl_2:hexane respectively, collecting the first 23 mL as eluate 1 and remainder as eluate 2 -conc. eluate 1 and eluate 2 -replace solvent with toluene/undecane	-GC/EC and GC/MS Eluate: 1. PCBs, PCNs 2. PCDD, PCDFs	Carp α / 100	92±7	94±3	98±6	104±4	95±8	99±22	91±16

α Data on salmon oil reported by Stalling et al. (1980) (see following page)
β Combined peak response used to calculate recovery.

Table II-1. (Cont'd)

Reference, Critique, Rating	METHOD; BASIC COMPONENTS				% Recovery Data[α]							
	Matrix	Extraction	Cleanup	Separation and Detection System	Spike ($\mu g.kg^{-1}$)	2,3,7,8-T_4CDD & T_4CDF	1,2,-4,7,-8-P_5CDF	1,2,-4,6-7,9-H_6CDF	1,2-3,6,-7,9-H_7CDD	1,2-3,4-6,7-8,9-H_7CDF	O_8CDD	O_8CDF
Stalling et al. (1980) -procedure previously discussed (Stalling et al. 1979; Smith 1980) Rating = 4	fish tissue 100 g	-extract fish by procedure of Stalling et al. (1979) -dilute oil, ca. 10 g/100 g fresh tissue, to 200 mg or less/mL with CH_2Cl_2 -spike with PCDF and PCDD congener mixture as follows: 1. 2.5 ng each in 10 g oil 2. 10 ng each in 20 g oil 3. 25 ng each in 10 g oil	-subject spiked extracts to following 4 chrom. column in tandem: 1.silica gel/KOH preparation 2.carbon/glass fiber 3.cesium silicate/H_2SO_4 silica gel 4.alumina Eluant: 1. 1, 10 mL hexane 2. 15 mL 2% CH_2Cl_2/hexane 3. 15 mL 5% CH_2Cl_2/hexane 4. 15 mL 8% CH_2Cl_2/hexane	-GC, 12 m OV-17 WCOT glass capillary column ^{63}Ni/ECD temp. program 190°C for 2 min, 4°C/min to 240°C for 15 min -2,3,7,8-T_4CDF and T_4CDD did not separate	Salmon Oil 25 100 250	 81±9 102±2 66±2	 70±5 97±3 80	 75±5 84±4 68±3	 82±3 98±2 76	 77±7 87±6 72±8	 87±7 76±5 66±3	 75±5 74±5 62±14

[α] n = 3 or 4
[β] Combined peak response used to calculate recovery.

Table II-1. (Cont'd)

Reference, Critique, Rating	METHOD; BASIC COMPONENTS				Detection Limits	Confirmation	VALIDATION OF METHOD			
	Matrix	Extraction	Cleanup	Separation and Detection System			Level of Fortification	N	% Recovery	± S.C. or C.V.
Kuroki and Masuda, (1978) -used method described by Nagayama et al. (1977) but extended GC columns and cited MS conditions in detail -limits of detection, quantitation not specified -used 9 PCDF isomers. Standard response linear over 0.02-0.10 $\mu g.mL^{-1}$ range for EC Rating = 5	human liver, adipose tissue 5-180 g	-homogenize with 150-400 mL hexane + 20 g anhydrous Na_2SO_4 -evaporate to dryness -reflux residue in 50 mL alcoholic N-KOH	-extract hexane -dry anhydrous Na_2SO_4 -conc. -silica gel chrom.; elute 150 mL hexane -conc. -activated alumina chrom. Eluant: 1. 60 mL 4:1 hexane:CCl_4 2. 20 mL 4:1 hexane:CH_2Cl_2 -replace eluant 2 with hexane for PCDF analysis -use eluate 1 for PCB analysis	-GC/EC 5 m x 3 mm i.d. 1.5% Apiezon L or 1.5% OV-17 or 4% SF-96 on chromosorb W AW DMCS 60/80 mesh isothermal 230°C -GC/MS 2 m x 2 mm i.d. 3% Apiezon L on chromosorb W AW DMCS 60/80 mesh	-	-GC/MS T_4CDF at m/e 304, 306; P_5CDF at m/e 338, 340; H_6CDF at 372, 374 -GC retention times on 3 different GC/EC columns	1-2 µg of 2,3,6,8; 2,3,7,8; 1,2,4,7,8; 2,3,4,7,8 PCDF in adipose tissue	-	85-90	-

Table II-1. (Cont'd)

Reference, Critique, Rating	METHOD; BASIC COMPONENTS				Detection Limits	Confirmation	VALIDATION OF METHOD			
	Matrix	Extraction	Cleanup	Separation and Detection System			Level of Fortification	N	% Recovery	± S.C. or C.V.
Buser et al. (1978a) -simplified clean-up procedure using micro columns -this procedure was applied to samples containing high levels of PCDF's i.e. 0.1 to 5.5 $\mu g.g^{-1}$ and may not be adequate in cleanup for lower levels such as low ppb and at ppt of PCDF's Rating = 4	Yusho oil, PCB oil 100 mg	-100 mg added to clean-up column	-0.5 g silica gel, 70-230 mesh Merck in 150 x 5 mm Pasteur pipette column -elute 6 mL hexane -concentrate hexane -1.0 g basic alumina in Pasteur pipette column Eluant: 1. 10 mL 2% CH_2Cl_2/ hexane for PCB's 2. 10 mL 50% CH_2Cl_2/ hexane for PCDFs and other tricyclic aromatic compounds -conc. to 50 µL for analysis	-GC/MS/EI; 50 m x 0.37 mm i.d. OV-17 capillary -obtained complete mass spectra using data system; optimal isomer separation using mass specific detection at m/e 270, 304, 308, and 372	-	-GCHR -selective mass specific separation	-0.1-1 $mg.kg^{-1}$ 2,3,7,8-T_4CDF added to rice oil and PCB; 2,3,7,8-T_4CDD; 2,3,6,7-tetrachloro-biphenylene	-	> 90	-

Table II-1. (Cont'd)

Reference, Critique, Rating	METHOD; BASIC COMPONENTS						VALIDATION OF METHOD			
	Matrix	Extraction	Cleanup	Separation and Detection System	Detection Limits	Confirmation	Level of Fortification	N	% Recovery	± S.C. or C.V.
Rappe et al. (1977) Rating = 4	Yusho oil	-see Nagayama et al. (1976) and Buser (1975)		-GC/MS; 22 m x 0.36 mm i.d. capillary column; OV-101 at 197°C; OV-17 at 207°C. Finnigan 1015 EI. 2 µL injections were used	-	-GC retention times, 2 columns -MS at congener m/e values -complete elution -mass spectra using Finnigan 4000 GC/MS with 6111 data system	-	-	-	-

Table II-1. (Cont'd)

Reference, Critique Rating	METHOD; BASIC COMPONENTS						VALIDATION OF METHOD			
	Matrix	Extraction	Cleanup	Separation and Detection System	Detection Limits	Confirmation	Level of Fortification	N	% Recovery	± S.C. or C.V.
Miyata et al. (1977) Rating = 4	human tissue, rice oil 10-20 g	-300 mL 1.5 N alcoholic KOH reflux 1 h + 100 mL H_2O	-extract 3 x 150 mL hexane -extract 2 x 200 mL H_2O dry anhydrous Na_2SO_4; conc. to 5 mL -Florisil column chrom. Eluant: 1. 100 mL hexane 2. 100 mL 5% EtOEt in hexane 3. 200 mL 50% EtOEt in hexane 4. 200 mL acetone -combine eluate 3 + 4, replace eluant with 3% CH_2Cl_2 in hexane -alumina column chrom. -elution of eluate 3 + 4 1. 200 mL 3% CH_2Cl_2 in hexane 2. 200 mL 20% CH_2Cl_2 in hexane (PCDFs) -see Miyata (1976) for remaining steps	-GC, 2 m x 3 mm OV-17 on Gaschrom Q, 240-280°C at 3°C/min, MS detection -Shimadzu LKB-9000 with MID	5 $\mu g.kg^{-1}$ for T_4CDF and P_5CDF	-GC retention times -MS of standards compared with extracts -MID fragments grams -loss of COCl, 2 Cl, etc.	-	-	-	-

Table II-1. (Cont'd)

Reference, Critique, Rating	METHOD; BASIC COMPONENTS						VALIDATION OF METHOD			
	Matrix	Extraction	Cleanup	Separation and Detection System	Detection Limits	Confirmation	Level of Fortification	N	% Recovery	± S.C. or C.V.
Nagayama et al. (1977) -see Nagayama et al. (1975) for detail Rating = 4	human liver, adipose tissue 5-180 g	-homogenize with 150-400 mL hexane + 20 g anhydrous Na_2SO_4 -evaporate to dryness -reflux residue in 50 mL alcoholic N-KOH	-extract hexane -dry anhydrous Na_2SO_4; conc. -silica gel chrom. elute 1 x 150 mL hexane; conc. -activated alumina chrom. Eluant: 1. 60 mL 4:1, hexane:CCl_4 2. 20 mL 4:1, hexane:CH_2Cl_2 -replace eluant 2 with hexane for PCDF analysis -use eluate 1 for PCB analysis	-GC/EC 2 m x 2 mm i.d. 2% SE-30 Chromosorb W AW-DMCS 100/120 mesh -GC/MS, Japan Electron Optics Lab. type D-100 with column as above. Monitored $C_{12}H_3OCl_3$; $C_{12}H_4OCl_4$; $C_{12}H_3OCl_5$; $C_{12}H_2OCl_6$	0.05 $\mu g.kg^{-1}$	-GC retention times -compare MS spectra of standard and sample extracts	1.0×10^2 $\mu g.kg^{-1}$	-	90	-

Table II-1. (Cont'd)

Reference, Critique, Rating	METHOD; BASIC COMPONENTS						VALIDATION OF METHOD			
	Matrix	Extraction	Cleanup	Separation and Detection System	Detection Limits	Confirmation	Level of Fortification	N	% Recovery	± S.C. or C.V.
Bowes et al. (1975a, b) -see also Bowes et al. (1973) Rating = 4	PCB, Yusho oil 1-2 g	-PCB dissolved in hexane, 400 mL -Yusho oil partitioned with acetonitrile system to recover PCB residues	-Florisil chrom. 180 g Eluant: 1. hexane, 1600 mL 2. 5% EtOEt/hexane, 800 mL 3. 25% EtOEt/hexane, 800 mL 4. acetone, 800 mL -alumina chrom. Eluant: 1. 1% $MeCl_2$/hexane 2. 20% $MeCl_2$/hexane	-GLC/EC; 3% OV-1 on 100/120 mesh Supelcoport, NiECD -trap effluent fractions -MS probe analysis, GEC AE1 MS 902, high resolution -GLC effluent fractions were trapped using a 20:1 effluent splitter and a U-shaped capillary tube immersed in liquid nitrogen	100 $ng.kg^{-1}$ oil or PCB	-GC retention time -MS spectroscopy comparison of standard and sample extracts	-	-	-	-

Table II-1. (Cont'd)

Reference, Critique, Rating	METHOD; BASIC COMPONENTS						VALIDATION OF METHOD			
	Matrix	Extraction	Cleanup	Separation and Detection System	Detection Limits	Confirmation	Level of Fortification	N	% Recovery	± S.C. or C.V.
Bowes et al. (1973) -no PCDF residues observed in eggs, liver or blubber Rating = 5	herring gull eggs, sea lion liver and blubber samples freeze dried for 5 days Clophen A-60, a German PCB preparation was used as a source of PCDFs for reference purposes	Egg, liver -grind with Na_2SO_4 -soxhlet extract with 1400 mL hexane/acetone azeotrope 8 h -concentrate and adjust volume to 1000 mL -determine lipid content on 3.0 mL volume Blubber -grind with Na_2SO_4 -soxhlet extract with 6300 mL of azeotrope mixtures for 4 days -conc. steps as for egg and liver PCB -5.50 mg dissolved in 400 mL hexane and added to Florisil clean-up column, etc.	-pass through 350 g Celite/H_2SO_4; conc. -Florisil chrom. 180 g Eluant: 1. hexane, 800 mL 2. 5% EtOEt/hexane, 400 mL 3. 25% EtOEt/hexane, 400 mL 4. acetone, 400 mL -exchange original solvent for hexane -alumina chrom. column for eluate 2, 3 and 4 -add hexane sample solution to column Eluant: 1. 1% CH_2Cl_2/hexane, 15 mL 2. 20% CH_2Cl_2/hexane -PCDOs and PCDFs are in acetone Florisil eluate and alumina 20% CH_2Cl_2/hexane eluate	-GC-^{63}Ni ECD, 3% OV-1 on 100-120 mesh Supelcoport 6' x 4 mm isothermal 200°C -individual peak components trapped in V-shaped capillary tube immersed in liquid nitrogen -low and high resolution MS/EI using GEC-AE1 MS-902 with XDS Sigma 7 computer	-	-GC retention times -probe MS spectra of eluted residue fractions trapped from GC -cleanup chrom. column characteristic elution pattern	-	-	-	-

APPENDIX III.0

TABULAR ABSTRACTS OF METHODS REVIEWED FOR FORMULATIONS

Table III-1. Methods reviewed for formulations.

Reference, Critique, Rating	METHOD; BASIC COMPONENTS				Detection Limits	Confirmation	VALIDATION OF METHOD			
	Matrix	Extraction	Cleanup	Separation and Detection System			Level of Fortification	N	% Recovery	± S.C. or C.V.
Buser (1975) -rapid micro procedure not isomer-specific but designed to separate into congener groups Rating = 4	PCB & TCP formulations 4 g	-dissolve in 30 mL MeOH -add 10 mL 2.5N LiOH; 100 mL H_2O -partition with 40 mL light petroleum (b.p. 40-65°C)	-wash petroleum fraction with mixture 100 mL dist. H_2O + 2 mL 2.5 N LiOH -wash with 50 mL dist. H_2O -dry over anhydrous Na_2SO_4 -conc. 20 mL aliquot to 2 mL -alumina micro column chrom. Eluant: 1. 10 mL hexane 2. 10 mL 50% CH_2Cl_2/ hexane 3. 10 mL CH_2Cl_2 -0.5 mL aliquots of eluted fractions conc. 5-10 times for GC/MS analysis -inject 10 µL representing 10-20 mg of original PCP or TCP	-GC/MS/EI Finnigan 1015D with a glass jet separator; 2 m x 2 mm i.d. glass column of 3% OV-1 on chrom. W AW DMCS -mass spectra recorded using temperature programming 140-260°C at 10°/min -mass fragmentry analysis at selected m/e values isothermally at 260°C -MS calibration using perfluorotributylamine and peaks at m/e 326, 376, 414, 426, 464 and 502 Eluates contained: 1. PCDPEs, PCB_{25}, PCBs 2. PCDDs, PCDFs 3. Phthalates	40 ng	-chrom. column separation characteristics -GC retention time -MS SID -Cl ion cluster ratio -fragment patterns -compare with standards	200 ng.kg^{-1} T_4CDD and H_6CDD 10 000 ng.kg^{-1} T_4CDD and H_6CDD	- -	85 95	-

Table III-1. (Cont'd)

Reference, Critique, Rating	METHOD; BASIC COMPONENTS				Detection Limits	Confirmation	VALIDATION OF METHOD			
	Matrix	Extraction	Cleanup	Separation and Detection System			Level of Fortification	N	% Recovery	± S.C. or C.V.
Curley et al. (1975) -qualitative screening only -chlorobiphenyl congeners present in fraction V and possible TCDF as indicated by Cl isotope cluster and m/z 304 Rating = 5	Aroclor 1254 rat urine 440 ml	-PCB; 1.7 g + 300 mL hexane -partitioned 6 x 50 mL of 3:1 EtOEt hexane -each 50 mL fraction centralized and supernatants combined -concentrate to 15 mL	-Florisil chrom. 180 g column; add samples Eluant: 1. 1200 mL hexane 2. 1200 mL 5% EtOEt/ hexane 3. 1200 mL 25% EtOEt/ hexane in 200 mL fractions III, IV, V, VII, VIII 4. 1000 mL 50% EtOEt/ hexane in 500 mL fractions IX, X -partitioned with acetonitrile by Mills procedure -methylated by Stanley procedure	-GC/MS; 6' x ¼" glass, 1.5% OV-17/1.95% QF-1, 60-80 mesh chrom. W, HP, AW, DMCS, 209°C; LKB-9000 MS, mass spectrum scan m/z 230-370	-	-GC/MS/EI retention times and m/z values compared with standard 2,3,7,8-TCDF	-	-	-	-

Table III-1. (Cont'd)

Reference, Critique, Rating	METHOD; BASIC COMPONENTS						VALIDATION OF METHOD			
	Matrix	Extraction	Cleanup	Separation and Detection System	Detection Limits	Confirmation	Level of Fortification	N	% Recovery	± S.C. or C.V.
Miyata et al. (1976) -interferences noted dibutyl phthlates (DBPs), PCBs, PCNs -heating KC-400 at 300°C in sealed ampuls, 15 days, did not alter PCB or PCDF content Rating = 4	Kaneclors (KC)- 300, 400, 500, 600	-0.5-1.0 g in 50 mL n-hexane	-Florisil chrom. 180 g -prewash with 500 mL hexane, add sample, rinse with 50 mL hexane Eluant: 1. 1700 mL n-hexane 2. 1000 mL 5% EtOEt/n-hexane 3. 1500 mL acetone -eluate 3 evap. to dryness; residue taken up in 3 mL $C_2H_2Cl_2$/n-hexane -alumina chrom. 20 g -prewash with 50 mL hexane; add sample Eluant: 1. 200 mL 3% $C_2H_2Cl_2$/n-hexane 2. 200 mL 20% $C_2H_2Cl_2$/n-hexane -eluate 2 analyzed for PCDFs	-GC/MS/EI; 2 m x 3 mm glass, 1.5% OV-17 on Chromosorb, 80-100 mesh -Shimadzu LKB-9000. Oven temp. program 200 at 3°C/min to 280°C -GC/EC; 1.8 m x 2 mm glass, 2% OV-17 on Gas Chrom. Q, 100-120 mesh; Varian 2100 GC; oven temp. program 180 at 4°C to 300°C	-	-GC/MS scan of mass spectra -retention times -PCDF standard preparation containing 46.3-56.8% chlorine -loss of COCl then 2 Cl for molecular ion	-	-	-	-

Table III-1. (Cont'd)

Reference, Critique, Rating	METHOD; BASIC COMPONENTS				Detection Limits	Confirmation	VALIDATION OF METHOD			
	Matrix	Extraction	Cleanup	Separation and Detection System			Level of Fortification	N	% Recovery	± S.C. or C.V.
Nagayama et al. (1976) Rating = 5	PCB formulations and rice oil	-oil, 5-10 g, saponify 1 h at 80°C with NaOH-ethanol -PCB formulations 0.1-0.5 g directly to alumina chrom.	-L/L partition with hexane -activated silica gel chrom. -elute 100 mL hexane conc. to 0.5 mL -alumina chrom. 5 g Eluant: 1. 20 mL hexane 2. 120 mL 20% CCl_4/hexane 3. 10 mL hexane 4. 30 mL 20% CH_2Cl_2/hexane -replace eluant 4 with hexane only -rechromatograph eluate 4 on 2 g alumina if PCB level too high	-GC/EC of isomers -perchlorination to O_8CDF -GC/MS, JEOL D-100 -no other detail given	-	-GC/MS spectra	1 µg per 10 g oil	-	90	-

Table III-1. (Cont'd)

Reference, Critique, Rating	METHOD; BASIC COMPONENTS						VALIDATION OF METHOD			
	Matrix	Extraction	Cleanup	Separation and Detection System	Detection Limits	Confirmation	Level of Fortification	N	% Recovery	± S.C. or C.V.
Rappe et al. (1978a) Rating = 4	Scandinavian PCP formulations	see Buser and Bosshardt (1976)		-HRGC/MS/EI; 50 m x 0.37 mm OV-17 or 50 m x 0.36 mm Silar 10C, temp. program 100-160°C at 20°/min, 2°/min to 250°C -Finnigan 4000 MS with 6111 data systems -m/z scan 35-500; MID at m/z 304, 338, 372, 406 and 440 -injected 2 uL equivalent to 4 mg of sample	-	-GC retention times -20 PCDF standards -mass fragmentography	-	-	-	-

APPENDIX IV.0

TABULAR ABSTRACTS OF METHODS REVIEWED FOR COMBUSTION SOURCES

Table IV-1. Methods reviewed for combustion sources.

Reference, Critique, Rating	METHOD; BASIC COMPONENTS						VALIDATION OF METHOD			
	Matrix	Extraction	Cleanup	Separation and Detection System	Detection Limits	Confirmation	Level of Fortification	N	% Recovery	± S.C. or C.V.
Stanley et al. (1982) -a guideline of sampling and analysis procedures for organic emissions -to complete the detail on methods it is necessary to have EPA Method 5, Federal Register, 41(111), 23060-23090 (1976) and EPA Method 613, Federal Register, December 1979, 69526-69531 Rating = 3	ash, filters, XAD-2 resin, coal, refuse-derived fuel aqueous samples	-soxhlet 8-24 h with benzene -grind coal to a powder prior to extraction (similarily for refuse) -dry all extracts using anhydrous Na_2SO_4 -the water-acetone-cyclohexane mix is shaken and the organic layer recovered; re-extract aqueous with 2 x 60 mL cyclohexane, combine and dry over anhydrous Na_2SO_4	-use procedure described in Stanley et al. (1982)	-HRGC/Hall-FID 30 m SE-54 -HRGC/MS-SIM fused silica Carbowax 20 M capillary, use PCDF m/z values of 202/204, 242/244, 270/272, 304/306, 338/340, 374/376, 408/410, 442/444	-	-HRGC/HRMS -other laboratory analysis -isotope labelled internal standards -reference samples	-	-	-	-

Table IV-1. (Cont'd)

Reference, Critique, Rating	METHOD; BASIC COMPONENTS						VALIDATION OF METHOD			
	Matrix	Extraction	Cleanup	Separation and Detection System	Detection Limits	Confirmation	Level of Fortification	N	% Recovery	± S.C. or C.V.
Liberti and Brocco (1982) Rating = 4	ash, gases, condensate	solids: -soxhlet 300 cycles with xylene	-silica gel chrom. eluant n-hexane -alumina chrom. Eluant: 1. 2% CH_2Cl_2 in n-hexane 2. 50% CH_2Cl_2 in n-hexane	-GC/EC; OV-17 and QF-1 on Supelcoport 100-120 mesh or 25 m OV-17 glass capillary column -GC/MS; VG 7070F, EI, m/e values for PCDDs: Tetra, 319.89, 321.89, 357.89; Penta, 353.85; 355.85, 357.85; Hexa, 387.81, 389.81, 391.81; Hepta, 421.78, 423.77, 425.77; Octa, 455.74, 457.73, 459.73 -PCDFs; above m/e values minus 16 -O_8CDD and O_8CDF overlap on GC	-	-GC retention times -3 mol. ions observed -characteristic fractionation pattern on chrom. column cleanup	-	-	-	-

Table IV-1. (Cont'd)

Reference, Critique, Rating	METHOD; BASIC COMPONENTS						VALIDATION OF METHOD			
	Matrix	Extraction	Cleanup	Separation and Detection System	Detection Limits	Confirmation	Level of Fortification	N	% Recovery	± S.C. or C.V.
Rappe et al. (1982) -abstract only: insufficient detail on methodology to rate	ash cement condensate soot	solids: -soxhlet 36-48 h with toluene	-multi-step incl. alumina chrom. -detail not given	-HRGC/MS	-	-HRGC/MS using standards for comparison	-	-	-	-

Table IV-1. (Cont'd)

Reference, Critique, Rating	METHOD; BASIC COMPONENTS						VALIDATION OF METHOD			
	Matrix	Extraction	Cleanup	Separation and Detection System	Detection Limits	Confirmation	Level of Fortification	N	% Recovery	± S.C. or C.V.
Liberti et al. (1982a) -see also Liberti and Brocco (1982) Rating = 4	urban incinerator water condensate and solid environmental samples	fly ash: -soxhlet 72 h with benzene 25 g conc. to 1 mL vapours: -trapped in ethylene glycol, then partions into CH_2Cl_2	fly ash: -chrom. column 2 g of silica (Merck kieselgel 60) -eluant 25 mL n-pentane, evaporate to dryness, take up residue in benzene or tetramethylbenzene for GC/MS PCB interference: -purify eluate from silica gel by alumina chrom. 1. elute with 2% CH_2Cl_2/hexane 2. 1:1, CH_2Cl_2:hexane for PCDDs and PCDFs -conc. for GC/MS	-HRGC; 25 m, OV-1 capillary column temp. program 25°C for 2 min, rapid rise to 190°C, hold 5 min, 2C°/min to 240°C, then 4C°/min to 270°C, interfaced directly to ion source inlet of VG 70-70F double focusing MS -LRGC; 2 m, 1.5% SP2250/1.95% SP2401 on 100/120 mesh Supelcoport, temp. program 200°C for 4 min, 2C°/min to 245°C, interfaced through a single-stage jet separator to the VG 70-70F MS -SID Unit; consisted of eight channels, monitored the molecular ion and two most intense isotopic peaks, $(M+2)^+$, $(M+4)^+$. PFK used as ref. mass resolution 3-5000	50-100 pg O_8CDF or O_8CDD on packed columns; few pg with capillary column	-GC retention times -SID m/z values -ratio of isotopic peaks	-not specified but consisted of O_8CDF, and T_4CDD, HCDD, O_8CDD	-	-	-

Table IV-1. (Cont'd)

Reference, Critique, Rating	METHOD; BASIC COMPONENTS[a]						VALIDATION OF METHOD			
	Matrix	Extraction	Cleanup	Separation and Detection System	Detection Limits	Confirmation	Level of Fortification	N	% Recovery	± S.C. or C.V.
Rappe et al. (1978c) -method used for other sample types (NRCC 1981b; App. II-9) Rating = 4	ash & gases from treated, then burned birch leaves and wood wool impregnated with commercial and purified chlorophenates. Smoke gases trapped on carbon or amberlite resin 10 mg chlorophenates 280°C for 30 min (micropyrolysis)	-carbon filter 15 g. Norit PK or amberlite XAD-2 resin -soxhlet 10 h using CH_2Cl_2	-silica gel chrom. 0.5 g micro column -elute 6 mL n-hexane -alumina chrom. 1 g Eluant: 1. 10 mL 2% $CHCl_2$/hexane 2. 10 mL 50% CH_2Cl_2/hexane -conc. eluate 2 and dissolve residues in 500 µL n-tetradecane inject 2 µl	-GC/MS; Finnigan 400 with EI source and 6111 data system; 50 m x 0.36 mm i.d. cap column, OV-17; temp. program 200-240°C, 2°C/min, mass specific detection molecular ions at m/e 320, 354, 388, 422 and 456 for T_4- to O_8CDD's; m/e 304, 338, 372, 406 and 440 for T_4- to O_8CDFs; two runs per sample were required -mass spectra runs m/e 35-460, 1.4 sec/scan using data system	0.01-0.20 $mg.kg^{-1}$ chlorophenate for T_4 and O_8CDF	-GC retention times -isotopic ration -clean-up column fractionation patterns -comparison with standards -substitution pattern of Cl	-	-	-	-

[a] See Buser (1975) for analytical procedure used with pure chlorophenate pyrolysis products.

Table IV-1. (Cont'd)

Reference, Critique, Rating	METHOD; BASIC COMPONENTS						VALIDATION OF METHOD			
	Matrix	Extraction	Cleanup	Separation and Detection System	Detection Limits	Confirmation	Level of Fortification	N	% Recovery	± S.C. or C.V.
Gizzi et al. (1982) Rating = 4	gas emission sampled as per specified trapping device	-collected at 0°C in isokinetic correlations 4 m^3 in 8 h -L/L partition of condensates and diethylene glycol trap 4 x following addition of 50 ng ^{37}Cl 2,3,7,8-T_4CDD	-conc. by evap. to dryness and dissolve residues in hexane -alumina chrom. Eluant: 1. 6 mL CCl_4 2. 4 mL CH_2Cl_2 -evap. eluate 2 to dryness and dissolve residue in 100 µL dioxane	-GC/MS; LKB 2091 with LKB 2130 computor. 2 m x 2 mm i.d. 3% OV-1 gas Chrom. Q. Monitored ions: T_4CDD m/z 320-322 P_5CDD " 354-356 H_6CDD " 388-390 H_7CDD " 422-424 O_8CDD " 458-460 T_4CDF " 304-306 P_5CDF " 338-340 H_6CDF " 372-374 H_7CDF " 406-408 O_8CDF " 442-444 -quantified on basis of standard response being equal for individual isomers -standards available ^{37}Cl-2,3,7,8-T_4CDD, 2,3,7,8-T_4CDD, 1,2,3,4-T_4CDD, 1,2,3,6,8,9-H_6CDD, O_8CDD, 2,3,7,8-T_4CDF, 1,2,3,4,7,8-H_6CDF, O_8CDF	-	-	-	-	-	-

APPENDIX V.0 A SUMMARY OF THE CURRENT APPROACHES TO SYNTHESIZING PCDF AND PCDD STANDARDS

V.1 INTRODUCTION

The references summarized below describe, in general terms, some of the reports giving reactions for preparing the individual PCDF isomers. The list does not encompass all published information but is meant to serve as an entry into the field.

Most authors caution against the possible health hazards associated with the compounds. Some indicate the need for special laboratory facilities and operating procedures. NRCC (1981b), Appendix VI.0, describes one facility that is appropriate for PCDF synthesis.

V.2 SUMMARIES

One of the early references to the preparation and use of authenticated standards was that of Bowes et al. (1975a). The authors used regiospecific methods and characterized the compounds by proton magnetic resonance and mass spectrometry. The synthesized standards were:

2,3,4-T_3CDF	2,3,6,7-T_4CDF	1,2,3,6,7-P_5CDF
2,3,6- "	2,3,6,8- "	1,2,3,7,8- "
2,3,8- "	2,3,7,8- "	2,3,4,6,7- "
1,3,6,7-T_4CDF	2,4,6,7- "	2,3,4,7,8- "
1,3,7,8- "	3,4,6,7- "	1,2,3,6,7,8-H_6CDF

A report by Gray et al. (1976) describes the synthesis of PCDDs and PCDFs. For PCDFs the authors used a modification of a diazotization and cyclization of the O-amino-polychlorodiphenyl ether process. Tetrachloroethylene was the solvent and isoamyl nitrite was the diazotizing agent in what was considered to be the most practical and general procedure. Isomers prepared were as follows:

2,3,8-T_3CDF	2,3,7,8-T_4CDF
2,4,6-T_3CDF	1,2,4,7,8-P_5CDF
1,2,7,8-T_4CDF	1,3,4,7,8-P_5CDF
2,3,6,8-T_4CDF	

Later, Gray et al. (1976) described their procedures for PCDFs in more detail and extended the number of isomers synthesized to include:

2,3,7-T_3CDF
2,3,9-T_3CDF
2,4,6,8-T_4CDF

Carbon-14-labelled 2,3,7,8- and 1,2,7,8-T_4CDF with specific activities of 57 mCi/mmol were synthesized by Gray et al. (1981). The major process consisted of Pschorr cyclization of O-phenoxyanaline-U ^{14}C, chlorination of the resultant dibenzofuran and separation of the tetrachloro isomers by HPLC.

Norström et al. (1979b) described the synthesis of five PCDF and three aminodibenzofuran isomers from the ring closure of chlorinated diphenyl ethers promoted by a $Pd(OAc)_2$ catalyst. The following compounds were prepared:

PCDFs; 3,7-D_2CDF
2,3,8-T_3CDF
2,3,7,8-T_4CDF
1,2,4,6,8,9-H_6CDF
2,3,4,6,7,8- "

Aminodibenzofurans; 2,6,9-Cl_3-4-NO_2
2,7,8-Cl_3-4-NO_2
4,7,8-Cl_3-2-NO_2

Synthesis of the higher chlorinated dibenzofurans using two separate routes has been described by Gara et al. (1979). One process used diaminodibenzofurans as intermediates but was seriously limited in its production of specific isomers. The other route relied on $Pd(OAc)_2$-promoted cyclization of chlorinated diphenyl ethers in a one step micro procedure followed by a simple purification step. The latter route was considered the more favorable process. Isomers prepared in yields of 30-60% at > 99% purity were:

2,4,6,8-T_4CDF
1,2,4,6,8-P_5CDF
2,3,4,6,8-P_5CDF
1,2,3,4,6,8-H_6CDF
1,2,4,6,7,8-H_6CDF
1,2,4,6,8,9-H_6CDF
2,3,4,6,7,8-H_6CDF
1,2,3,4,6,7,8-H_7CDF

Some phenols and diphenyl ethers were also prepared.

Development of a set of isomer standards, including fully labelled UL-^{13}C PCDFs, is considered an essential component of GC/MS method development by Bell (1983). The author describes the synthesis of ^{13}C-labelled 1,2,3,4- and 2,3,7,8-T_4CDFs using uniformly ^{13}C-labelled phenol, benzene and UL-^{13}C benzene reagents. Higher PCDFs were obtained by chlorination of the 2,3,7,8-^{13}C-T_4CDF in an antimony pentachloride/carbon tetrachloride reaction. Mixtures of various isomers were obtained.

The synthesis of all 38 T_4CDF congeners was reported by Mazer et al. (1983). The reaction processes consisted of pyrolysis of specific polychlorinated biphenyl congeners (Buser and Rappe 1979), ultraviolet photolysis of pentachlorodibenzofurans and chlorination of

specific trichlorodibenzofurans by electrophilic aromatic substitution. Identification of the isomers per se was based on reaction specificity in combination with capillary column chromatography and mass spectrometry detection. The relative retention times (2,3,7,8-T_4CDF) were determined and are listed in Table 3-4 of this publication. The authors used RP-HPLC to separate 10-50 µg amounts of single isomers from chlorinated reaction mixtures.

Safe and Safe (1983) synthesized 22 mono to hepta homologues by the base-catalyzed cyclization of their corresponding hydroxy-PCB precursors. The hydroxy-PCBs were synthesized from their methoxy analogs using either the diazo coupling of chlorinated anisidines and symmetrical chlorinated benzenes or the diazo coupling of chlorinated anilines and chlorinated anisoles.

Table V-1 summarizes with reference sources the single, isolated isomers that have been synthesized to the present. These data do not include other isomers known to be in mixtures that are useful in the identification and confirmation process (e.g. Bell (1983) has reported 1,2,6,7,8-; 1,2,3,8,9-P_5CDF; 1,2,3,7,8,9-H_6CDF and 1,2,3,4,7,8,9-H_7CDF).

Table V-1. Summary of PCDF isomers reported isolated or synthesized as separate identities (data after Bowes et al. 1975b; Gray et al. 1975, 1976; Buser 1976a; Kuroki and Masuda 1978; Rappe et al. 1978a, c; Stalling et al. 1979b; Norström et al. 1979b; Gara et al. 1979; Mazer et al. 1983; Safe and Safe 1983).

Mono(4)	Di(16)	Tri(28)	Tetra(38)		Penta(28)	Hexa (16)	Hepta (4)	Octa(1)
1	3,7	2,3,8	2,3,6,8	1,3,6,9	1,2,4,7,8	1,2,3,6,7,8	1,2,3,4,6,7,8	1,2,3,4,6,7,8,9
2	3,6	2,4,6	2,3,7,8	2,3,4,9	1,3,4,7,8	2,3,4,6,7,8	1,2,3,4,6,8,9	
3	2,8	2,3,7	1,2,7,8	1,2,3,4	2,3,4,7,8	1,2,4,6,8,9	1,2,3,4,6,7,9	
4	2,3	2,3,9	2,4,6,8	1,2,3,8	1,2,3,7,8	1,2,3,4,6,8		
	2,6	2,7,8	1,2,8,9	1,4,6,7	1,2,3,4,8	1,2,4,6,7,8		
	2,7	2,3,6	1,3,6,8	1,2,3,6	1,2,3,6,7	1,2,4,6,7,9		
		2,3,4	1,3,7,8	1,3,4,9	2,3,4,6,7	1,2,3,4,7,8		
		2,6,7	1,3,7,9	1,2,6,7	1,2,6,7,8	1,2,3,4,6,7		
		1,3,8	1,3,4,7	1,2,7,9	1,2,4,6,8			
		1,3,6	1,4,6,8	1,4,6,9	2,3,4,6,8			
			1,2,4,7	1,2,4,9				
			1,3,6,7	2,4,6,7				
			1,3,4,8	2,3,4,7				
			1,3,4,6	1,2,3,9				
			1,2,4,8	1,2,6,9				
			1,2,4,6	2,3,4,8				
			1,2,6,8	2,3,4,6				
			1,2,3,7	2,3,6,7				
			1,4,7,8	3,4,6,7				

APPENDIX VI.0

CRITIQUE AND RECOMMENDATIONS FROM:
POLYCHLORINATED DIBENZO-p-DIOXINS:
LIMITATIONS TO THE CURRENT ANALYTICAL TECHNIQUES
NRCC #18576. NRCC (1981b)

1.2 CRITIQUE

The Panel's comprehensive examination of methodology revealed that development has been intensive and great progress has been made in the 1970's. Thus, detection limits for PCDDs in chemical products have developed from 1000 $\mu g.kg^{-1}$ in 1969 to 1 $\mu g.kg^{-1}$ in 1980. Similarly, these detection limits for 2,3,7,8-T_4CDD in environmental samples have been lowered from 50 $\mu g.kg^{-1}$ in 1970 to 1 $ng.kg^{-1}$ in 1978. Furthermore, the ability to determine specific isomers in a particular homologue and separate them from all other congeners has advanced from the ability to separate 2,3,7,8-T_4CDD from only two of its isomers in 1974 to an ability to distinquish it from all of its 21 isomers in 1978. Likewise all 10 isomers of H_6CDD have been separated as have the two isomers of H_7CDD.

Such dramatic and effective evolution of methodology for the determination of specific compounds in a series of homologous compounds is unprecedented in the history of chemistry and was made possible only by the continuous investment in the best equipment and manpower of government agencies, academia, and industry working together as a team.

As the methodology advanced so did the demands on the analytical chemists. So that, in spite of the advances of analytical science, several weaknesses have persisted and often still exist. Two identified by the Panel are the lack of data indicating sampling procedures and the validation of residue extraction efficiency. This situation weakens the reliability of much of the analytical data on PCDD residue levels in various matrices.

The procedures used generally do not provide a homogeneous representative sample, but only what is essentially, a grab sample with no definable statistical basis. The results are then simply characteristic of the sample analyzed and not necessarily the bulk of the matrix being considered. Exceptions include the collection and pooled sampling of gull eggs by Canadian Wildlife Service and the Dow protocol for analysis of fish near their plant in Michigan. Exhaustive extraction of samples and comparative assessment of the different techniques, extractants, etc., have been evaluated, in part, by the Dioxin Implementation Plan but the data have not been published in their entirety. Even with adequate sampling regimes, it is still difficult to accurately determine the efficiency of an extraction procedure. Validation of the remaining sequences in the analytical methods is more extensive since techniques involving fortification with native PCDDs and/or internal standards have been used.

Recoveries obtained in various laboratories for native T_4CDDs at concentration levels of 0.5 to 10 $ng.kg^{-1}$ in biological matrices have varied greatly (from 25±7% to 86±7%, uncorrected). It has been the practice to use internal standards such as $^{37}Cl_4$-T_4CDD and $^{13}C_{12}$-T_4CDD to correct for losses inherent in the method. There is some question about the use of the internal standard technique in some applications,

particularly when the levels of labelled material exceed the amount of T_4CDD present by 50 to 2500 times. While there should be less of a problem in determining the homologues H_6CDD, H_7CDD and O_8CDD at the generally high levels in formulated materials, such concerns may arise when determining these congeners at the very low levels expected in some environmental matrices. This is particularly true when an isomer of one homologue such as 1,2,3,4-T_4CDD is used as a standard for another homologue such as O_8CDD. The response factor will vary between the congeners and could result in an underestimation of the quantity present by as much as 10-fold. The usually accepted limit of quantitation at 2-3 times the limit of detection is clearly not applicable. In this respect the methodology needs validation. Particulates from combustion sources present a specific problem. The fortification of uncontaminated material does not provide a valid test of the extraction efficiency, and approaches to assess extraction techniques for bound residues have not yet been developed.

Interlaboratory precision has been sometimes good, sometimes only fair. One must not expect to obtain a tight, narrow range of values when analyzing for ultra-trace levels in complex matrices. The coefficient of variation is expected to increase considerably with a decrease in residue concentration. This is particularly true in studies between laboratories. At the $ng.kg^{-1}$ level of analysis, the expected coefficient of variation can greatly exceed ±60% and is independent of the determinative method. This is not surprising in view of the very minute amounts that must be separated quantitatively from the vast amount of matrix by a series of complex chemical and physical sequences. The implication of these observations is that it will be exceedingly difficult to obtain high levels of precision and accuracy at the levels of PCDDs being encountered in environmental matrices. It will be necessary to establish the practical limits of precision and accuracy where PCDDs are concerned.

The ability of the present "state of the art", using LRMS detection to resolve, unequivocally, one homologue from another, has been demonstrated with a good degree of surety for most matrices. This is not true for their isomers. Only recently (1979-81) has it been possible to separate 2,3,7,8-T_4CDD from the 21 other isomers and the 10 H_6CDD isomers from each other. Such techniques are not generally developed for the other homologues. It is important to realize that most other procedures described for T_4CDDs have the potential to determine, as one identity, two or more isomers in the cleanedup[α] extract of a sample. This means that there is often a great deal of uncertainty concerning the presence of a specific T_4CDD isomer such as 2,3,7,8-T_4CDD. The complete separation technique must be validated before conclusions can be drawn.

[α] Cleanedup is used to indicate that the sample has passed through a cleanup procedure.

Confirmation procedures rely heavily on both the mass spectrometer and the resolving capabilities of different capillary GC columns. If the criteria described are followed, one can have a reasonable degree of confidence that positive observations indicating T_4CDD are correct. Despite this, it is possible to have false positives under certain circumstances with compounds such as chlorinated methoxybiphenyls or tetrachlorophenyl benzoquinone. Clearly there is a need for more exhaustive examination for other potential interferences particularly to validate extensive use of LRMS as the final determinative step. However, HRMS will not be superceded but remains essential to analysis when a high degree of certainty is required, such as for regulatory purposes.

The complexity, difficulty and incomplete state of validation studies associated with present methods of PCDD analysis raise some questions concerning the usefulness of some of the work to date and, specifically, the conclusions drawn from the residue data. Several authors have described their validation studies and referred to their participation in a quality assurance program. These results have not yet been reported in full and are not presently available for examination. Further, as indicated by the ranking procedure used herein, there remain a considerable number of methods being used to produce data whose value is severely restricted when used to interpret the status quo.

A major factor limiting research is the shortage, or lack, of individual standards for all isomers of all the homologues. Sufficient quantities of the T_4CDDs do not exist to meet the needs of analytical chemists, let alone for meaningful metabolic and toxicological studies required to determine their relative activities. At present, standards are restricted to a few milligrams of specific isomers such as 2,3,7,8-T_4CDD or pyrolytic mixtures. Most data accumulated to date concern 2,3,7,8-T_4CDD. Toxicological evaluations for other isomers (previously available only as mixtures) appear to be largely based on hypothetical assumptions related to spatial distribution of the chlorine atoms within the different isomers. These uncertainties make it difficult to assign a value to the required limits of detection. Data tabulated in the appendices indicate that it is possible to achieve a lower limit of 0.5 to 1.0 $ng.kg^{-1}$ with quantitation at 10 $ng.kg^{-1}$ or greater. What remains to be clarified, by toxicologists, is whether these are adequate performance values or whether it is necessary to continue to increase sensitivity.

Today, the current methods of bioassay lack any degree of homologue, yet alone isomer, specificity or sensitivity as compared with traditional methodology. The bioassay methods are developmental and require extensive validation before their usefulness as screening tools is proven. They could potentially serve as integrators of the combined toxicity of PCDDs and work in this direction should be encouraged.

Other factors affecting our ability to accumulate scientific data relating to PCDDs are the unavailability of equipment, trained personnel, and special laboratory facilities to deal with the acutely toxic nature of many of the PCDD congeners. Mass spectrometers with ancillary equipment, including data acquisition and processing, are expensive. When associated with complex chemical analysis directed to obtaining very reliable data to satisfy litigation, monitoring or research needs, the rate of sample output is severely restricted; for example, the Dow protocol requires three days to process a single human milk sample. This high degree of confidence is required because the data generated are subject to being made sensational by the media. False positive results are particularly touchy. In most situations, such a high degree of confidence may be inappropriate. For instance, in determining the gross scope of a contamination problem it is advantageous first to screen for a positive result and later to confirm the presence of specific isomers in s[illegible] samples.

Overriding these lati[illegible] restrictions are the budgetary considerations that keep staffing and operational funds at an inadequate level to provide the equipment, personnel, and special laboratories needed to carry out meaningful surveys.

1.3 RECOMMENDATIONS

From the tone of this critique, obviously much could be done to improve our analytical capabilities vis-à-vis PCDDs. Throughout the text, we have made numerous specific comments applicable to specific matrices. There is an overall concern on the part of the Panel that there is a lack of perspective as to the meaning of results being generated today. As a first step to improving the situation, we recommend the following:

- It should be mandatory that the release of data on PCDD be accompanied by a statement as to the limitations on interpretation of the data and the extent and rigor of the validation procedure.

- It is necessary that a central source of PCDD standards, potential interfering compounds (e.g. pre-dioxins) be established in North America and international exchange be allowed for scientific purposes.

- With sufficient funds, the detection limits of various PCDDs can be improved. Before undertaking such an exercise, toxicologists should define the requirements for analytical capabilities.

- Work should continue on the bioassay techniques in order to answer questions about the correlation between the results determined through the classical chemical techniques and their actual biological implications.

- Given the sensitive nature of the results obtained, laboratories should participate in interlaboratory validation studies.

- There should be quality assurance programs monitored by an independent scientific committee.

- The use of PCDDs in all types of laboratories should be thoroughly and formally examined and a series of appropriate safety protocols should be devised.

APPENDIX VII.0

TECHNICAL ASSESSMENT AND RECOMMENDATIONS FROM:
POLYCHLORINATED DIBENZO-p-DIOXINS:
CRITERIA FOR THEIR EFFECTS ON MAN AND HIS ENVIRONMENT
NRCC #18574 NRCC (1981a)

1.2 TECHNICAL ASSESSMENT OF THE SCIENTIFIC CRITERIA

Text Section

Prologue

The large number of high quality publications on the subject of polychlorinated dibenzo-p-dioxins attest to the significant advances made in the fields of chemical analysis and toxicology during the past decade. It is estimated that the cost of these studies in the United States alone could have amounted to at least 100 million dollars. As a result, the information on 2,3,7,8-tetrachlorodibenzo-p-dioxin far exceeds that normally available to assess the environmental impact of other high-priority pollutants. It is also now evident that the phenoxy herbicide 2,4,5-T is not the only source of 2,3,7,8-tetrachlorodibenzo-p-dioxin and that far greater quantities of many of the other 74 polychlorinated dibenzo-p-dioxins are entering the environment from a variety of sources. Many researchers have shifted their interests to these other polychlorinated dibenzo-p-dioxins, as well as to the polychlorinated dibenzofurans, although our understanding of the scientific criteria is still far from complete.

Delineating the magnitude of the input to the environment

We have speculated from the limited data available that as much as 1.5 tonnes of polychlorinated dibenzo-p-dioxins could be entering the Canadian environment each year. 2.1.5

The major identified sources are associated with the use of chlorophenols in the wood industry, but considerable quantities also enter the environment from the combustion of organic materials. The contribution from chemical dumps is unknown but they could be point sources of local contamination. 2.1.1 2.1.2 2.1.4

The present use pattern of herbicides, such as 2,4,5-T and Silvex, does not contribute a significant input to the environment. However, 2,4-D is a significant source of di-, tri- and tetrachlorodibenzo-p-dioxins although the polychlorinated dibenzo-p-dioxins found in 2,4-D are not thought to be particularly toxic. 2.1.1 2.1.5 Table 1-2

The total amount of polychlorinated dibenzo-p-dioxins entering the Canadian environment cannot be firmly established because representative levels in most Canadian products reaching the market place have not been fully delineated. However, the extensive use of pentachlorophenol accounts for the fact that octachlorodibenzo-p-dioxin constitutes more than 3/4 of the total loading of polychlorinated dibenzo-p-dioxins from recognized sources. 2.1.2

Significant amounts of the highly toxic hexachlorodibenzo-p- 2.1.1

dioxins and heptachlorodibenzo-p-dioxins also enter the environment from the use of chlorophenols and as products of combustion. Section 2.1.2

The role of combustion

While the role of combustion as a potential source of numerous polychlorinated dibenzo-p-dioxins is now largely accepted, the thermal conditions which maximize their rate of production are unknown. Precursors of dibenzo-p-dioxins are known to be formed after simply adding hydrogen chloride to the flame of a Bunsen burner. There are now a large number of independent observations on the formation of polychlorinated dibenzo-p-dioxins from a variety of combustion processes, including the burning of fossil fuels, of wood in wood stoves and of cigarettes. Most investigations have centered on the municipal incineration of garbage and many of the polychlorinated dibenzo-p-dioxin homologues have been found. The amount of polychlorinated dibenzo-p-dioxins emitted varies considerably, depending probably on the type of material burnt and also the operating conditions. Residues are being detected in Canadian fly ash but only the nature of the homologues has been confirmed. The precise quantities of the homologues as well as the isomer content of Canadian sources are not known. However, on the basis of a study on a United States municipal installation, as much 2,3,7,8-tetrachlorodibenzo-p-dioxin may enter the Canadian environment from combustion sources per year as ever entered during the period of maximum annual use of the phenoxy herbicide 2,4,5-T. While the paucity of information does not allow any firm conclusion to be drawn, the predominant attention given to 2,4,5-T as a source must be questioned. 2.1.3 2.1.2 2.1.2 2.1.5

Residues in environmental matrices and their detection

Very few data exist regarding the levels of polychlorinated dibenzo-p-dioxin residues in the Canadian environment, particularly data that can be related to the human situation. Residues of polychlorinated dibenzo-p-dioxins have been reported in Canada in fish and chicken livers. It is not known whether the results are characteristic of the average contamination or are artifacts of the very limited and biased sampling programs. At present, one must rely almost entirely on the reports from other countries in order to make inferences about Canadian residues. 2.2

The validity of any residue data is dependent on the analytical methodology, therefore a comprehensive examination of analytical methodology was carried out. Three App. II

of the most serious weaknesses identified by that Panel are: Section
a) the lack of validated sampling procedures for the matrix,
b) the lack of comprehensive studies on the efficiency of extraction procedures and
c) the unavailability of appropriate standards.
The Panel found: "that the procedures generally used do not provide a homogeneous representative sample of the matrix, but only, what is essentially, a grab sample with no definable statistical basis." Although there are notable exceptions, the results are then simply characteristic of the sample analyzed and not necessarily representative of the bulk of the matrix being considered. Exhaustive extraction of samples and comparative assessment of the different techniques, extractants, etc., have been evaluated, in part, but much of the data have not yet been published. It is particularly difficult to determine accurately and with confidence the efficiency of an extraction procedure.

The authors of that report also pointed out that App. II
while the homologue content can be determined with confidence, the precise nature of the polychlorinated dibenzo-p-dioxin isomer can, with few exceptions, be determined with much less confidence. The Panel stated that: "The ability of the present 'state of the art', using Low Resolution Mass Spectrometry to resolve, unequivocally, one homologue from another has been demonstrated with a good degree of surety for most matrices. This is not true for their isomers as only recently has it been possible to separate 2,3,7,8-tetrachlorodibenzo-p-dioxin from the 21 other tetra isomers, the 10 hexachlorodibenzo-p-dioxin isomers and the 2 heptachlorodibenzo-p-dioxin isomers. Such techniques are not generally developed for the other homologues. It is important to realize that most other procedures described for tetrachlorodibenzo-p-dioxin have the potential to determine, as one identity, two or more isomers in the cleaned-up extract of a sample. This means that there is often a great deal of uncertainty concerning the presence of a specific tetrachlorodibenzo-p-dioxin isomer such as 2,3,7,8-tetrachlorodibenzo-p-dioxin." It is necessary to examine the validation of the complete separation technique before conclusions can be drawn.

Confirmation procedures rely heavily on both the App. II
mass spectrometry and the resolving capabilities of different capillary gas-liquid chromatographic columns. When the procedures are applied exhaustively, one can be reasonably confident that positive observations indicating tetrachlorodibenzo-p-dioxin are correct. However, despite extensive cleanup, false positives can be obtained. Potential interferences have been studied, but not exhaustively. The ultimate confirmation is obtained by

thorough isomer separation coupled with the use of High Resolution Mass Spectrometry. Obviously, this cannot be applied to every sample but until there is a better understanding of the potential for interferences, the application of High Resolution Mass Spectrometry to the final extracts will be required when a high degree of certainty is needed, such as for regulatory purposes. Section

As a consequence, most early claims about 2,3,7,8-tetrachlorodibenzo-p-dioxin contamination are in doubt, and even more recent claims are suspect. In addition to the limitations arising from the analytical techniques, only limited samplings of the various matrices have been completed. Consequently, we cannot be entirely certain as to the extent and level of contamination, which systems are contaminated, or whether polychlorinated dibenzo-p-dioxins, other than 2,3,7,8-tetrachlorodibenzo-p-dioxin, are major contaminants. In the Great Lakes, 2,3,7,8-tetrachlorodibenzo-p-dioxin has been reported as the predominant polychlorinated dibenzo-p-dioxin although this belies the fact that usually it was the only one being sought. When the detection of others has been attempted, others have usually been found. App. II App. I 2.1.3.3

As the analytical capability for detecting lower and lower amounts of environmental pollutants has progressed, each successive breakthrough has been heralded with a rash of reports. The implication has been that the residues did not exist prior to detection and that some new risk to humans is now imposed. Often the conclusions are inadequately supported. Detection does not imply a risk per se for it is the degree of exposure that determines the toxic response. The nervousness that has been created by the premature release of data is regrettable since there is now an over-reluctance to release data, even though it has been exhaustively substantiated. This interferes with the free exchange of information and advancement of our understanding.

Even with all the aforementioned reservations there is now sufficient evidence to suggest that, at very low levels, polychlorinated dibenzo-p-dioxins, including 2,3,7,8-tetrachlorodibenzo-p-dioxin, could be ubiquitous contaminants in North America. It is not known whether the presence of 2,3,7,8-tetrachlorodibenzo-p-dioxin as a residue is related primarily to the manufacture of 2,4,5-trichlorophenol and its derivatives, or to combustion sources. Without an understanding of the processes governing the persistence and fate of polychlorinated dibenzo-p-dioxins, and hence their accumulation, sources of contamination cannot be established with any confidence from existing programs which monitor the levels of polychlorinated dibenzo-p-dioxins only in biota such as fish and piscivorous birds. 2.2 3.3 3.4 6.2.4

In the design of future monitoring programs one must recognize that, as with polychlorinated biphenyls (PCBs) and other persistent organic chemicals, concerns are not only with the general contamination patterns but also with the presence of localized hot spots which can result from the spillage or dumping of contaminated materials such as fly ash, oils, wood preservatives, disinfectants and herbicides. The multiplicity of potential sources in every developed part of Canada means that the specific local use and disposal patterns of contaminated chemicals must be carefully documented to identify which areas deserve first attention. Section

Human safety and the toxicological criteria

Pragmatically, environmental contaminants are significant only in their relationship to human safety or, more generally, to environmental quality as it may be perceived for human needs or desires. The risk to any organism resulting from exposure to polychlorinated dibenzo-p-dioxins must be judged with respect to the toxicological criteria: death, debilitation, or reduced reproductive ability. The most clearly recognized effects to be considered are the short-term, or acute, lethality of polychlorinated dibenzo-p-dioxins; long-term effects such as their potential to promote cancer; immunosuppression that can result in increased susceptibility to infection and possibly death; and interferences with reproductive processes, resulting in reduced reproductive potential or handicapped offspring (teratogenesis). A clear picture is beginning to emerge with respect to the toxicological effects of 2,3,7,8-tetrachlorodibenzo-p-dioxin in laboratory animals. Toxicologic data for all other polychlorinated dibenzo-p-dioxins are lacking with respect to all of these effects. 5.1

A summary of the data pertaining to lethality, carcinogenicity and reproductive effects is presented in Table 1-2. The bulk of the data suggests that the polychlorinated dibenzo-p-dioxins are not mutagenic. 5.5
Immunosuppressive effects have been demonstrated, but only after short-term, relatively high exposure studies with 5.6
2,3,7,8-tetrachlorodibenzo-p-dioxin. These data are equivocal and contradictory in terms of criteria for assessing potential hazard. The various congeners show a Table 1-2
wide range of lethality, differing from the "super toxic" Table 5-1
2,3,7,8-tetrachlorodibenzo-p-dioxin, to the apparently "practically non-toxic" octachlorodibenzo-p-dioxin. Considerable variation in the sensitivity of different species is observed with the congeners tested to date. In the case of 2,3,7,8-tetrachlorodibenzo-p-dioxin, they vary from 1 $\mu g.kg\text{-}bw^{-1}$ in the guinea pig to 5000 $\mu g.kg\text{-}bw^{-1}$ (dietary) in the Syrian hamster. The reasons for these

Table 1-2. Summary of the dosage associated with the toxicological criteria for various PCDD congeners (to two significant figures).

Homologue	Isomer	Acute Lethality				AHH Induction	Carcinogenicity (rat)		Reproductive & Teratogenic (rat)			
		Range of LD_{50} Vertebrates ($\mu g.kg\text{-}bw^{-1}$)	LD_{50} Guinea pig[β] ($\mu g.kg\text{-}bw^{-1}$)	Relative to[β] 2,3,7,8-T_4CDD Guinea pig	Mouse	Relative to[ε] 2,3,7,8-T_4CDD (rat hepatoma cells)	Minimum effect ($\mu g.kg\text{-}bw^{-1}.d^{-1}$)	No observed effect ($\mu g.kg\text{-}bw^{-1}.d^{-1}$)	Birth effects	Embryotoxic effects	No teratogenic effect ($\mu g.kg\text{-}bw^{-1}.d^{-1}$)	No reproduct effect
D_2CDD	2,7-	>2,000,000[α]				33,000					100,000[η]	
	2,8-	300,000[β]	>300,000	>150,000		33,000						
T_3CDD	2,3,7-	30,000[β]	30,000	15,000	>1500	3100						
T_4CDD	2,3,7,8-	2-5000[βγ]	2	1	150	1	1×10^{-2}[ζ]	1×10^{-3}[ζ]	1×10^{-1}[η]		3×10^{-2}[η]	$1 - 2 \times 10^{-3}$[η]
	1,3,7,8-					240						
	1,2,3,8-					1700						
	1,2,3,4-	>1,000,000[δ]				33000					800,000	
P_5CDD	1,2,3,7,8-	3-338[β]	3	1.5	170	15						
	1,2,4,7,8-	1100-5000[β]	1100	560	>2500	330,000						
H_6CDD	1,2,3,4,7,8-	70-800[β]	73	40	410	21	36×10^{-2}[θ]		100[α]	1-10[α]	1×10^{-1}[α]	
	1,2,3,6,7,8-	70-1300[β]	70-100	40	630	85						
	1,2,3,7,8,9-	60-1400[β]	60-100	40	>720	130						
	1,2,3,6,7,9-					220,000						
H_7CDD	1,2,3,4,6,7,8-	>600-[β]	>600	>300		370						
	1,2,3,4,6,7,9-					10000						
O_8CDD		>1,000,000[α]				53000			500,000[α]			

[α] Schwetz et al. (1973).

[β] McConnell et al. (1978a).

[γ] Olson et al. (1980b), Henke et al. (1981).

[δ] Saint-Ruf (1978).

[ε] Bradlaw and Casterline (1979).

[ζ] Section 5.3.1.2.B.

[η] Section 5.4.

[θ] Section 5.3.1.2.C, a mixture of 31% 1,2,3,6,7,8-H_6CDD and 67% 1,2,3,7,8,9 (percent of total H_6CDD)

differences are not entirely understood, but as will be seen, studies on the mechanisms of action may help to explain them. 5.7

Interpretation of the toxicological criteria with respect to the residue levels and analytical detection limits

The data for the other toxicologic criteria, carcinogenicity and reproductive effects are much less complete than the data on lethality (Table 1-2). Only 2,3,7,8-tetrachlorodibenzo-p-dioxin has been studied in any detail although the carcinogenic potential of a standard mixture of 31% 1,2,3,6,7,8- and 67% 1,2,3,7,8,9-hexachlorodibenzo-p-dioxin administered to rats and mice has been studied. These criteria are of greatest concern from the viewpoint of the analytical detection limits required for residue monitoring, particularly as they pertain to human exposure and risk. The exposure level demonstrating no reproductive effect in rats of 1 ng 2,3,7,8-tetrachlorodibenzo-p-dioxin.kg-bw^{-1}.d^{-1} (Table 1-2) is equivalent to a total daily intake of 60 ng 2,3,7,8-tetrachlorodibenzo-p-dioxin for a 60 kg woman. Intakes in this range would be encountered when the average concentration in the total diet reaches 30 ng.kg^{-1} (assuming a daily food intake of 2 kg). The principal identified food item in the Canadian diet, for which concern has been expressed over 2,3,7,8-tetrachlorodibenzo-p-dioxin contamination, is fish from Lake Ontario. Given a meal of 120 g (about 0.25 lb), intakes of 60 ng would be encountered when fish containing 500 ng 2,3,7,8-tetrachlorodibenzo-p-dioxin.kg^{-1} are consumed daily. This assumes that no other food items are contaminated. Given the possibly ubiquitous nature of this contaminant, it is not necessarily a prudent assumption.

5.3.1.2.A
5.3.1.2.B
App. II
6.0
5.4
App. I
6.2
6.2

The dose of 2,3,7,8-tetrachlorodibenzo-p-dioxin observed to have no detectable carcinogenic effect in rats was virtually the same as that observed to have no reproductive effects (Table 1-2). Superficially, the same argument could apply. However, a number of extrapolative techniques have commonly been used in the case of carcinogenicity because of the uncertainty as to whether there is a no-effect level. While it has been argued that such extrapolations provide a conservative basis for criteria, their application to the human situation remains unproven. Nevertheless, these extrapolative techniques are being used to define acceptable levels of risk by evaluators (e.g. a "Virtually Safe Dose" for a predicted increase of 1 cancer in a million individuals). For 2,3,7,8-tetrachlorodibenzo-p-dioxin, the most useful "Virtually Safe Dose" obtained from these extrapolative techniques appears to be in the range of 2 to 10 fg 2,3,7,8-tetrachlorodibenzo-p-dioxin.kg-bw^{-1}.d^{-1} or

5.3.4
5.3.4.4

between 2 and 6 pg.d^{-1} over the lifetime of a 70 kg individual. These values are about 10,000 times lower than those developed for the reproductive effects. Section

In terms of food consumption, the average concentration of 2,3,7,8-tetrachlorodibenzo-p-dioxin in the daily diet of a 70 kg human should be only 1-5 pg.kg^{-1}, based on these extrapolations. If one were concerned with fish as the only contaminated portion of the total diet, a daily intake of 120 g (0.25 lb) should, on this basis, contain only 20-50 pg.kg^{-1} of 2,3,7,8-tetrachlorodibenzo-p-dioxin. By accepting 1 cancer per 10,000 individuals, and if fish were eaten only once a week, the acceptable concentration of 2,3,7,8-tetrachlorodibenzo-p-dioxin would be 14-35 ng.kg^{-1} fish, i.e. just above the 3-30 ng.kg^{-1} limit of quantitation, or 2-3 times the limit of detection. Evaluations based on the no observed carcinogenic effect level in rats, such as the recent assessment of risk made by United States and Canadian regulatory agencies, are consistent with the "Virtually Safe Dose" predicted at a risk level of 1 cancer in 10,000 individuals. App. II App. I 5.3.4.4

Thus, if only fish are contaminated, if only 2,3,7,8-tetrachlorodibenzo-p-dioxin is of concern, and if only reproductive effects are critical, our analytical capabilities are just adequate. On the other hand, if the whole diet is assumed to be contaminated, the criteria for reproductive effects yield dietary exposure levels at or quite near today's limits of quantitation and thus offer no margin for error. But, if one uses the criteria obtained for carcinogenicity and employs the extrapolative techniques together with the more conservative risk levels, our analytical capabilities are inadequate for detection, let alone quantitation with respect to any procedure for evaluation.

The question of man's sensitivity and its resolution through structure-activity relationships

At this point, it is correct to question the applicability of the scientific criteria for carcinogenicity to the human situation. Polychlorinated dibenzo-p-dioxins are so clearly associated with man's way of life through his use of fire for heating and cooking that it can be suggested that man has developed resistance to polychlorinated dibenzo-p-dioxins. If man is atypical with respect to other organisms, the extrapolative problems may not be real. The Panel concludes that the hypothesis of man's atypical sensitivity deserves as much attention as does the further improvement of the analytical detection limits.

It is the Panel's opinion that the resolution of some of these concerns may best be obtained through further examination of the fundamental structure-activity relations reflected in the specific receptor binding and the extent of enzyme induction (such as aryl hydrocarbon hydroxylase) as they pertain to toxic effects. The progress made in this area in less than a decade is demonstrated by the parallels that have been elucidated between the acute toxicity of the various polychlorinated dibenzo-p-dioxin congeners and:
(a) their potency in inducing aryl hydrocarbon hydroxylase activity;
(b) the coincident genetic segregation of mouse sensitivity with aryl hydrocarbon hydroxylase activity.
Additionally, a soluble cytoplasmic (cytosol) receptor protein for 2,3,7,8-tetrachlorodibenzo-p-dioxin has been discovered to which other polychlorinated dibenzo-p-dioxins bind competitively. How these mechanisms and observations are related to the ultimate cause of death and man's sensitivity is as yet unknown, but promises to be a highly fruitful area of research.

Section 5.7

The structure-activity hypothesis has been only partially tested although it is generally a good indicator of the relative lethality of the polychlorinated dibenzo-p-dioxin congeners. There are notable exceptions. Thus, based on aryl hydrocarbon hydroxylase induction, 1,2,4,7,8-pentachlorodibenzo-p-dioxin would not be expected to be as lethal as either octachlorodibenzo-p-dioxin or 2,3,7-trichlorodibenzo-p-dioxin, whereas in fact, it is significantly more lethal to guinea pigs than either of the other two (Table 1-2).

Of the eleven congeners tested for acute lethality (Table 1-2), seven, including 2,3,7,8-tetrachlorodibenzo-p-dioxin, can be classified as "super toxic" even though some may be 1000-fold less toxic than 2,3,7,8-tetrachlorodibenzo-p-dioxin. 2,3,7-Trichlorodibenzo-p-dioxin can be classified as "extremely toxic" while 2,7-dichlorodibenzo-p-dioxin, 2,8-dichlorodibenzo-p-dioxin and octachlorodibenzo-p-dioxin can be considered "practically non-toxic".

Table 1-2
Table 5-1

Some doubt can be raised about the non-toxic nature of octachlorodibenzo-p-dioxin. Its low solubility suggests that the vehicles used in oral dosing studies could result in its inefficient sorption and hence the results could be artifacts of the experimental protocols. Such might not be the case when natural matrices (e.g. tissue) are encountered.

Table 3-7

Fate and Persistence

Section

As with many other chlorinated hydrocarbons, polychlorinated dibenzo-p-dioxins are highly persistent in the environment. The only major removal process from any situation found in the environment for which sound theoretical arguments exist, is volatilization. Polychlorinated dibenzo-p-dioxins do photolyze in the presence of hydrogen donors; however, the relevance of this observation in most natural systems is not known because information is lacking on the role and abundance of hydrogen donors. In aquatic systems such processes would be limited since light is attentuated as the depth increases. On soil surfaces, processes involving photolysis have been used for rapid decontamination (2,3,7,8-tetrachlorodibenzo-p-dioxin has a half-life of a few hours). However, material more deeply buried in soils could have a much longer half-life, exceeding 10 years. The levels of 2,3,7,8-tetrachlorodibenzo-p-dioxin in the eggs of herring gulls from the Lake Ontario basin have progressively shown a 50% reduction every 2 years over the past decade. The reasons for this decline are unknown because of the paucity of information on the basic processes affecting the fate of polychlorinated dibenzo-p-dioxins in such systems. We cannot yet determine the relevance of these observations to the higher homologues detected.

3.0

3.2.2

3.2.1

There is no documentation concerning the persistence of either chlorophenols or polychlorinated dibenzo-p-dioxins in treated wood. However, chlorophenols, in their undissociated state, should volatilize more readily than the polychlorinated dibenzo-p-dioxins; in fact, volatilization may be the only operative removal process for polychlorinated dibenzo-p-dioxins from wood. Whether long-term preservation arises, in part, from the properties of the polychlorinated dibenzo-p-dioxins is also unknown. However, when entrained in wood, polychlorinated dibenzo-p-dioxins are bioavailable. For example, swine and poultry, when housed in chlorophenol-treated wooden pens or on chlorophenol-treated litter, have been found contaminated with polychlorinated dibenzo-p-dioxins.

NRCC (1981b)

6.2.2

The ultimate sinks for polychlorinated dibenzo-p-dioxins include aquatic systems. Organisms which frequent such systems, including piscivorous birds and mammals, deserve particular attention since they could serve as indicators of potential problem areas. There is a paucity of useful criteria for assessing the impact of polychlorinated dibenzo-p-dioxins on organisms associated with aquatic systems. Limited studies suggest that levels of 2,3,7,8-

3.4

tetrachlorodibenzo-p-dioxin, similar to those that induce reproductive problems in rats, also affect the reproductive potential of birds. Lethality of fish has been estimated to occur at levels below 700 $\mu g.kg\text{-}bw^{-1}$. The studies are, however, too limited to form a basis for sound criteria. Section 4.4 4.5

Bioaccumulation and man's exposure to hazard

High levels of polychlorinated dibenzo-p-dioxins can be potentially accumulated by fish. In the case of birds and their eggs, polychlorinated dibenzo-p-dioxins are most likely obtained from foods. Piscivorous birds may have a slight tendency to accumulate polychlorinated dibenzo-p-dioxins to higher levels than the fish they eat and are therefore potential accumulators. Their reproductive status, as well as that of fish, could be an indicator of the general health of a system, vis-ã-vis the effects of polychlorinated dibenzo-p-dioxins. When effects are detected it would seem prudent to examine whether health effects can also be detected in humans. Epidemiologically, there is no conclusive proof of any effects of polychlorinated dibenzo-p-dioxins on the general population. The possible exception is the accident at Seveso where significant increases in the incidence of chloracne and neurological damage have been observed. 6.2.2 6.2.4 3.4 5.3.3

Because of man's use of products contaminated with polychlorinated dibenzo-p-dioxins and his continual exposure to by-products of combustion, he is the prime receptor of concern. Thus, it is important to define the exposure encountered by Canadians by virtue of their occupation or their daily activities. Certainly, the exposure encountered by workers in the wood industry and by those who apply wood preservatives needs to be closely documented to determine if a risk exists. In the absence of Canadian studies the Panel has closely examined information from other countries to identify possible exposure patterns of concern. Whether these parallels pertain to Canada has yet to be established. 6.3 6.3.1 6.3.2

6.2.2

A few samples of Canadian foods other than fish have been examined and found to contain polychlorinated dibenzo-p-dioxins, although their precise nature is not well characterized. We do not know whether these residues are representative of the background contamination being encountered or are simply associated with unrepresentative problems in the food industry. It is also not known whether the detection of chlorophenol residues in a variety of foods is also indicative of polychlorinated dibenzo-p-dioxin contamination. 2.2

While providing no solid basis for developing risk assessments of the dietary vector, these residues do indicate that there is a need to monitor foods, other than fish, if the Canadian situation is to be characterized. Other foodstuffs that clearly deserve attention include vegetables grown or stored in chlorophenol-treated materials and meat from animals raised in treated pens and on contaminated litter such as wood shavings. While the use of contaminated materials (e.g. chlorophenol treated wood), as an agricultural practice, has been restricted, the effectiveness of this program should be determined through monitoring.

Section 2.2, 6.2.2

Because of the extremely low solubility of the polychlorinated dibenzo-p-dioxins in water, potable water is not the primary vector of concern. In most situations, the dermal and dietary vectors present a potentially greater hazard to the general public than does the water vector. In certain occupational situations, inhalation may be important. The dermal and inhalation toxicity of these compounds has received little attention. The relevance of the toxicology, developed primarily with dietary studies, to these situations remains largely unknown.

Section 3.0, 5.3

The laboratory use of polychlorinated dibenzo-p-dioxins requires formal examination to assess what is an adequate safety protocol. While those concerned with the analysis of polychlorinated dibenzo-p-dioxins have directed much attention to the hazards posed by the handling of the more toxic congeners, very toxic polychlorinated dibenzo-p-dioxins, particularly 2,3,7,8-tetrachlorodibenzo-p-dioxin, are used extensively as positive controls in many biochemical, physiological and toxicological studies. In these situations there has been little discussion of the associated hazards.

NRCC (1981a)

1.3 RECOMMENDATIONS

The Panel was charged with the task of developing and assessing the usefulness of the scientific criteria for polychlorinated dibenzo-p-dioxins and of identifying critical gaps in our knowledge. The available criteria are insufficient to provide anything but a cursory assessment of the polychlorinated dibenzo-p-dioxin problem, albeit our understanding of 2,3,7,8-tetrachlorodibenzo-p-dioxin is sufficient for preliminary assessments of the toxicological implications associated with the detection of these chemicals in foods and products available to Canadians. It is impractical to repeat the process for each of the remaining 74 polychlorinated dibenzo-p-dioxins. Setting aside economic considerations, the manpower requirements associated with such a project are beyond our capabilities.

THE PANEL RECOMMENDS THAT EMPHASIS BE GIVEN TO STUDIES AIMED AT ELUCIDATING THE BASIC PATTERNS COMMON TO THE 75 CONGENERS RATHER THAN STUDIES OF NARROW SCOPE WITH A SINGLE COMPOUND.

PRIORITY I

(A) THE DETERMINATION OF MAN'S SENSITIVITY TO POLYCHLORINATED DIBENZO-p-DIOXINS COMPARED WITH OTHER MAMMALS.

PURPOSE: To establish the validity of extrapolative techniques applied to the criteria on carcinogenic and reproductive effects of polychlorinated dibenzo-p-dioxins such that assessments can be made on:

(i) the current level of polychlorinated dibenzo-p-dioxins with respect to the health of man;
(ii) the adequacy of the current analytical capabilities to provide an acceptable margin of human safety;
(iii) the effects of combinations of polychlorinated dibenzo-p-dioxins to which humans are being exposed.

(B) THE CONTINUED MONITORING AND ASSESSMENT OF THE LEVELS OF POLYCHLORINATED DIBENZO-p-DIOXINS ALREADY IN THE ENVIRONMENT WITH FOCUS ON MATRICES CLOSELY ASSOCIATED WITH MAN (e.g. FOOD) AND WITH FOCUS ON THE IDENTIFICATION OF SOURCES.

PURPOSE: To determine:

(i) the extent and background levels of polychlorinated dibenzo-p-dioxins;

(ii) man's exposure, with particular reference to the identification of critical groups potentially at risk;
(iii) the relative importance of sources of polychlorinated dibenzo-p-dioxins in the Canadian context so that the effectiveness of potential remedial actions can be assessed.

Special criteria requirements related to monitoring:

The development of a fate model and associated criteria which describe the processes influencing the accumulation and persistence of the various polychlorinated dibenzo-p-dioxins in environmental matrices. The objective should be to identify general indicators of contamination for it is presently impossible to relate the results of the monitoring surveys to input vectors and sources.

PRIORITY II

DETERMINATION OF THE RELATIVE TOXICITY OF THE VARIOUS POLYCHLORINATED DIBENZO-p-DIOXINS TO FISH, WITH PARTICULAR ATTENTION TO POSSIBLE REPRODUCTIVE AND IMMUNOSUPPRESSIVE EFFECTS.

PURPOSE: Fish are primary accumulators of polychlorinated dibenzo-p-dioxins and scientific criteria are needed to assess at which levels of contamination fish stocks are endangered.

RECOMMENDATIONS FOR SCIENTIFIC CRITERIA WITHIN THE CONTEXT OF POTENTIAL REMEDIAL ACTIONS

A number of pragmatic remedial actions could be taken which would lessen man's exposure and hence our need for additional research would be reduced to a more manageable level. There are basically three pragmatic actions available to the regulator. Assuming that the human situation is to be assessed, each action necessitates the development of additional criteria. Significantly more criteria are needed if regulatory actions are not taken.

ACTION 1 - RESTRICTION OF PCDD INPUTS FROM COMBUSTION SOURCES

PURPOSE: The reduction of inputs associated with sources that have led and will continue to lead to the wide distribution of polychlorinated dibenzo-p-dioxins in the Canadian environment.

Specific information and studies associated with Action 1 that would be required include:

(i) An improved understanding of the incineration process such that methodology can be developed to minimize polychlorinated dibenzo-p-dioxin emissions.

(ii) An Inventory of combustion sources in Canada and adjacent States in the United States to determine the quantities of polychlorinated dibenzo-p-dioxins emitted and the relative importance of various combustion sources so that priorities can be established for remedial actions.

(iii) The determination of whether polychlorinated dibenzo-p-dioxins, entrained in matrices associated with combustion, are available to biota to allow an assessment of the toxicological significance.

ACTION 2 - RESTRICTION OF THE POLYCHLORINATED DIBENZO-p-DIOXIN INPUT FROM THE USE AND MANUFACTURING OF CHLOROPHENOLS

PURPOSE: To eliminate the known major polychlorinated dibenzo-p-dioxin sources and thus:

(i) reduce the number of occupational and domestic exposure situations that need to be assessed;

(ii) reduce the need for dermal and inhalation toxicology of polychlorinated dibenzo-p-dioxins such that they would no longer be a priority subject requiring immediate attention.

CHLOROPHENOL-OPTION 1: THE BANNING OF ALL USES OF PENTA- AND TETRACHLOROPHENOLS

Specific requirements for additional information and studies include:

An Inventory of uses in order to design a monitoring program to determine the effectiveness of the action with particular emphasis on the identification of situations where the period between the introduction of a remedial measure and the cessation of input is long, e.g. leaching from dumps.

CHLOROPHENOL-OPTION 2: THE REPLACEMENT OF TECHNICAL GRADE PENTACHLOROPHENOL WITH TETRACHLOROPHENOL OR PURIFIED PRODUCTS

This option has received some attention. It would reduce the polychlorinated dibenzo-p-dioxins present in the parent material and hence the risk in certain occupational and domestic groups. The counter arguments are that purified pentachlorophenol administered to pregnant rats imposed an greater teratogenic risk to the fetuses than did the technical pentachlorophenol. While purified and technical tetrachlorophenol are less fetotoxic than pentachlorophenol, the technical tetrachlorophenol contains trichlorophenol, and hence there is the possibilty of additional environmental inputs of 2,3,7,8-tetrachlorodibenzo-p-dioxin. Furthermore, at least on treated surfaces, the same polychlorinated dibenzo-p-dioxins are formed by the action of light on the chlorophenols.

To demonstrate whether this is a viable alternative will require:

(a) The determination of the rates at which polychlorinated dibenzo-p-dioxins are formed from chlorophenols under environmentally relevant conditions and the rates at which polychlorinated dibenzo-p-dioxins are destroyed under similar conditions to clarify whether there would be a net decrease in the polychlorinated dibenzo-p-dioxin input.

(b) A mass balance of 2,3,7,8-tetrachlorodibenzo-p-dioxin entering the environment from the use of tetrachlorophenol.

Specific requirements for additional information and studies for option 2 include:

(i) The determination of the effects of the formulations on the reproductive potential of mammals exposed through the dermal and inhalation vectors.

(ii) The development of an Inventory of production processes and related waste streams to determine whether polychlorinated dibenzo-p-dioxins enter the environment as by-products of the purification process.

(iii) As Option 1 above.

ACTION 3 - RETENTION OF THE STATUS QUO

RESULT: In the Panel's opinion, retention of the status quo will continue the input of large quantities of polychlorinated dibenzo-p-dioxins - a situation where the concerns expressed in the critique remain unchanged and the need for comprehensive criteria which permit the assessment of combinations of polychlorinated dibenzo-p-dioxins will become increasingly acute. These could take decades to develop and the continued inability to adequately assess the situation will, in the long-term, inevitably lead to increased criticism and uneasiness.

In this case the Panel recommends that additional scientific criteria be obtained which permit assessments to be based upon:

1. A thorough understanding of which Canadians are exposed to the highest levels of polychlorinated dibenzo-p-dioxins, which combinations of polychlorinated dibenzo-p-dioxins are encountered by these groups and the level and duration of the exposures associated with the various routes of intake.

2. A thorough understanding of how the carcinogenic, reproductive and immunosuppressive effects of these

toxicants are manifested when man encounters combinations of polychlorinated dibenzo-p-dioxins through not only the dietary vector but also the inhalation and dermal vectors.

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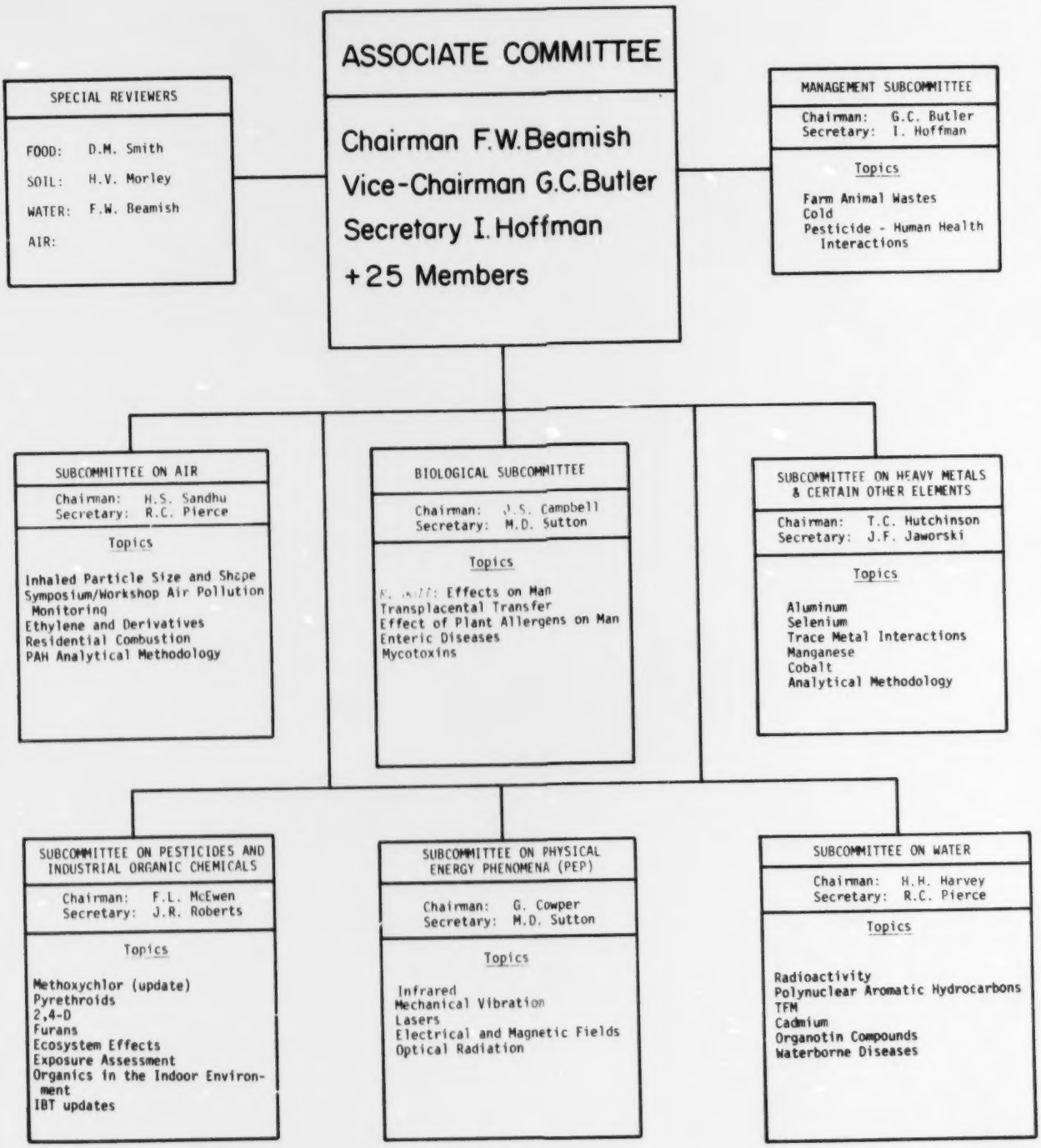
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